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A SURVEY OF THE RH HAPTEN

BETTINA B. CARTER[a]

Since an inhibitor for Rh antibody derived from the Rh positive red cell was first described in 1947 (1), much has been learned concerning its nature. At first, this fraction was given as a therapeutic measure to the erythroblastotic newborn. Then it was decided to use it as a preventive by providing it for the pregnant woman. Nothing was understood as to dosage or frequency or timing. These things had to be learned by trial and error. The first pregnant woman to receive the Rh hapten had 100 mg. once every two weeks! Much later, a pregnant woman received 400 mg. daily. Originally, it was thought that a few administrations just prior to delivery might be beneficial. Now we know that a sensitized woman must begin treatment before she begins a new pregnancy. For many years the Rh hapten was given intramuscularly. Now it has been demonstrated that oral administration is even more effective.

"Hapten" is a term which was coined by Landsteiner to indicate a substance which is not in itself a complete antigen, but which is antigenic when coupled with a protein and which will by itself neutralize specific antibody. The fraction called Rh hapten fits this category. It is not antigenic, but can be made so by loose combination with such substances as ovalbumin and it will act to neutralize Rh antibody, either in the test tube or in vivo. It is produced, as described previously (2, 3, 4, 5), by pooling Rh positive human red cells, of any blood group, precipitating the proteins with alcohol and then extracting the lipid hapten from the precipitate with dichloromethane or ether. It produces no reactions, either systemic or local, when injected or when taken orally as observed in quantities used to date (up to 600 mg. daily). It is lipid, with very little carbohydrate, and gives no tests for protein, but does contain as much nitrogen as would be expected in a crude substance containing phospholipid. It superficially resembles petroleum jelly in appearance. When injected into rats, rabbits, guinea pigs or humans, it is not antigenic, but may be readily made so for the first three species by protein coupling. It will adsorb nitrogen as antibody from Rh anti-sera and this can be measured quantitatively (6).

The Rh hapten may be given to an Rh negative woman at any time upon initial discovery of sensitization and, if she has no history which includes erythroblastotic infants, the success is 98 per cent, as ascertained to date over a twelve year period. If the hapten is provided a previously sensitized Rh negative woman with a homozygous husband, a history of erythroblastotic losses, an antibody titer against Rh as determined by two independent laboratories and provision of the hapten is made before a new pregnancy is initiated, success is 100 per cent, as determined over a four-year period. Details of these findings will be reported elsewhere.

Several interesting findings have come to light in the search for answers as to what the Rh hapten can and cannot do. We know, for instance, that the fraction has no therapeutic properties. It will cure nothing. However, if used early, it apparently will combine with antibody and prevent damage to the fetal red cell in utero. Also we know that a baby of a treated mother, no matter how thoroughly the mother has been treated, will show a positive Coombs test at birth unless the mother has received the hapten throughout delivery. We realize also that it is beneficial for the new born to be given the Rh hapten throughout the first week of life.

Time is on the side of the Rh negative woman treated with Rh hapten. Less treatment is required in inverse proportion to the length of time elapsed since the birth of a previous affected infant. Also, if the mother is treated, it is preferable that the pregnancy be allowed to go to term as opposed to early induction of labor. There have been no cases of kernicterus with babies from mothers treated with Rh hapten.

If at least a year has elapsed since the last delivery, the response of the Rh antibody titer is prompt, seldom requiring longer than three months with 100 mg. daily given orally. But if treatment is attempted during the first year after a delivery, the tide of antibody rise is very strong and it may require the full year to bring the antibody titer to zero. This might be overcome by massive dosage, but it has not been attempted as yet. Antibody titrations using plasma as a diluent will demonstrate a fall in titer, but it is difficult, using other diluents, to show any effect on titer at all. This substantiates Chown's (7) observation that the nature of antibody titration is not yet understood. At present, the

woman receives 100 mg. daily until her titer is zero. Her doctor advises pregnancy and she is given from 200 to 300 mg. daily throughout pregnancy.

This review has attempted to bring our understanding of the use and value of the Rh hapten up to date.

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HORMONES IN OBSTETRICS AND GYNECOLOGY

L. C. Powell, Jr. and Garth L. Jarvis

Probably the employment of hormones is one of the most abused and poorly understood in the field of Obstetrics and Gynecology. One can scarcely pick up any journal today without finding some article on this subject and many drug advertisements about the efficacy of treating some condition with hormones. If we believe all of these, we would have few problems to deal with in our practice. Intelligent use of hormones is very difficult unless one has a basic understanding of the physiology of menstruation and reproduction and uses this knowledge in hormonal therapy. Since hormonal treatment is replacement therapy, there should be sound basis by either adequate diagnostic criteria and studies or astute clinical judgment for the use of hormones. The hormone should either replace the product of a hypofunctioning organ or inhibit the secretion of an overactive gland. One must remember the action of the various hormones before using these medications.

In the normal physiology of menstruation, we know there is a delicate balance and interplay of the hormones secreted by the pituitary, ovary, thyroid, and adrenal. Some of these are fairly well understood and other still elude us. In the normal menstrual cycle, Follicle Stimulating Hormone (FSH) from the pituitary acts to stimulate the cells in the ovarian follicle to secrete estrogen during the follicular phase. Estrogen promotes uterine growth, proliferation of the endometrium, and inhibition of FSH secretion. The pituitary then releases Luteinizing Hormone (LH) and Luteuotrophic Hormone (LTH). LH initiates and LTH maintains the secretion of progesterone from the corpus luteum. Progesterone produces secretory changes in the endometrium and inhibits secretion of LH and LTH. It must be remembered, however, that progesterone action on the endometrium depends on prior estrogen stimulation, but not vice-versa. Exogeneous estrogen does not act directly on the ovary, but any change in ovarian function is produced directly through the pituitary by reduction of the FSH. Estrogen, however, does cause stimulation of body cells in the same manner as physiologic estrogen (1). The func-

tions of the thyroid and the adrenal secretions in this hormonal physiology is still unknown except for the fact that their proper function is essential for the regularity and predictability of the cycle. The knowledge of this hormonal physiology in clinical practice and the availability of newer and more powerful synthetic preparations has given new meaning to the rational use of hormones. However, we still have no clinically effective gonadotrophic hormones perfected as yet for use in human therapy.

Menstrual Disorders

Menstrual disorders are among the most common symptoms seen by the obstetrician-gynecologist. Therefore, it is not unusual that this subject should be the first to be categorized and discussed. Ovarian function, due to either functional or anatomic obliteration, as seen in the menopausal syndrome, is characterized by an ovary that ceases to respond to stimulation. This removes the normal brake on the pituitary and causes an excess secretion of FSH and the ovary is unable to secrete estrogen and progesterone. Thus, menstruation stops and the uterus atrophies. Replacement therapy with estrogen may be logically expected to help regulate chemical, physiological, and psychological homeostasis, but, of course, restoration of ovulation is impossible. Does estrogen, in fact, restore these functions? It is felt that estrogens are specific for the vasomotor symptoms of ovarian deficiency and we have fairly good proof of its ability to alter protein and osseous metabolism. This is probably the best reason for treating the young castrate empirically. Psychological benefit is dubious in our opinion, but perhaps this is because we know so little of the psyche.

In the woman with the uterus intact, there is a continuous threat of bleeding from a hyperplastic endometrium with continued estrogenic therapy. Androgen or progesterone in sufficient dosage tends to counteract this bleeding effect through end organ response. Androgen or progesterone also increases the vasomotor response level. Thus combinations of estrogen with androgen or progesterone are effective in long term therapy. Dosage, in general, is individualized and the effects of therapy can be judged by the subjective relief of hot flashes or in the laboratory with vaginal smears and in difficult cases by FSH levels. The latter is

quite expensive and usually not necessary. Usually 1–3 mg. of stilbestrol (or its equivalent) each day is sufficient to control hot flashes. In addition to the use of testosterone to counteract the bleeding of continued estrogen administration there is a metabolic need for testosterone in the aged or young castrate to aid in the prevention or arrest of osteoporosis. The ratio of about 1 mg. of stilbestrol (or equivalent) to ten to fifteen mg. of testosterone, has been found to be the most efficacious in proper balance of sex end organ and metabolic balance. The only clinical effect as to rejuvenation in the elderly person and prolonging life by prophylactic sex hormone administration is a boost in the "general feeling of well-being." This is such a nebulous thing that we consider this in the same category as the "shot" working better than the pill.

The greatest abuse of hormonal therapy in the menopause, which we see far too often, is hormonal therapy in patients with irregular bleeding without proper investigation as to cause. The danger of uterine malignancy in this age group must escape some physicians or surely they would not be "passing a pill" without even a cursory examination, much less a thorough pelvic examination, along with Papanicolaou smears and appropriate biopsies or curettage. The physician, who gives hormones to the menopausal patient without regard to her intense need for reassurance and emotional support so necessary in handling the emotionally insecure patient, is missing perhaps the most important facet of the menopausal syndrome. This is only adding fuel to the fire and not extinguishing it.

Causes of Amenorrhea may be divided into several categories (2):

1. Mechanical blockage.
2. Organic deficiency—absence or destruction of any of the organs concerned.
3. Physiologic—as pregnancy or menopause.
4. Organic pathology in the reproductive system—such as polycystic, fibrotic ovaries (Stein-Leventhal) and pituitary tumors.
5. Organic pathology outside the reproductive system—myxedema, Addison's disease, adrenogenital syndrome, masculinizing tumors, such as arrhenoblastoma and adrenal secreting tumors.

6. Functional—e.g. Psychic stress.
7. Apparent amenorrhea-periodic microscopic bleeding.
8. Unknown etiology.

Amenorrhea always must be thought of as a symptom and not a diagnosis. Amenorrhea, due to endocrine disturbances has the basic abnormality of anovulation and failure of progesterone secretion. However, the cause of amenorrhea should be searched for, as hypofunction of the thyroid (borderline or real) and the adrenal gland may be the etiologic factor in this symptom complex and replacement therapy should be with thyroid or corticosteroids. This may be corrective without resorting to the use of female sex hormones.

Adrenal hyperfunction may also cause amenorrhea in Cushing's disease or in the adrenogenital syndrome. The basic defect in the adrenogenital syndrome is failure of hydrocortisone synthesis. ACTH is secreted by the pituitary gland in excessive amounts and stimulates androgen production by the adrenal glands. FSH production is reduced, and estrogen is lacking. Administration of hydrocortisone inhibits ACTH, and gonadotrophic hormone is secreted in normal quantities to produce a regular cycle of menstruation.

Careful history and physical examination should lead one to perhaps place the cause of amenorrhea into one or more of the above categories or exclude it. Diagnostic workup should include basal body temperature records, serial vaginal smears and/or fern tests, trials of bleeding response to progesterone, frequent pelvic examinations and endometrial biopsies. Thus, it is possible to pinpoint the category to either apparent, functional, or of unknown type.

Frequently you will find some emotional element present like the patient with pseudocyesis, but in the usual case, symptoms are associated with some change in the patient's total life situation. These patients will bleed when treated with 25–50 mg. of progesterone. Edometrial biopsy will show proliferative, mild atrophic, or hyperplastic endometrium.

The uterus may be small if there is persistant anovulation, with the unresponsive uterus giving a negative progesterone bleeding test and little or no endometrium on biopsy.

The principle of treatment in the functional type should be to correct the anovulation if possible. Psycho therapy of the super-

ficial ventilatory type may be corrective over the long term but can be aided markedly by cyclic therapy. Single doses of progesterone, 25–50 mg. at 28 day intervals or 10–50 mg. for three to five days is usually sufficient. Basal body temperatures and endometrial biopsies should be used to judge response of therapy. If there is a poor bleeding response, a full artificial cycle with estrogen and progesterone may be needed. In most cases, cycling should be done for a three month period. In some cases, cortisone, 15–25 mg. per day, has been beneficial in this type of amenorrhea, as well as in some cases of Stein-Leventhal and adrenogenital syndrome. Again, it should be emphasized that it is the exception, rather than the rule to be able to pinpoint any specific case in one office visit.

Dysfunctional uterine bleeding or more commonly called functional uterine bleeding is another symptom that may be due to disturbed hormonal secretions. This condition occurs most frequently at the menarche or the menopause. The hormonal picture is usually like that of anovulation, or poor ovulation, with bleeding from a poor or non-secretory endometrium. The menstrual cycle is characteristically that of an irregular irregularity. This same type of bleeding may occur when estrogen is given continuously for an indefinite time or in constant insufficient amounts. A reduction in effectiveness occurs and the subsequent bleeding will be irregular in type, frequency, and amount. Like amenorrhea, however, other causes should be investigated, such as disorders of blood clotting, local conditions; such as tumors (benign or malignant) and pregnancy (abnormal, usually).

The key diagnostic procedure is uterine curettage properly timed to be done on the first day of bleeding or the day before, always remembering the possibility of aborting a pregnant patient. The uterine curettage may be omitted in the teenager unless response to therapy has been poor. Endometrial biopsy may be used in lieu of uterine curettage but should not be used if a malignancy is possible as then curettage is mandatory. Treatment is aimed to control the present bleeding episode and to establish a normal cyclic bleeding pattern in the future. Many times uterine curettage itself accomplishes this by breaking the vicious cycle of physiologic response to the hormonal imbalance. Hormonal therapy can be aimed at this same effect (the so-called medical curettage) by giving the patient 50 mg. of progesterone,

thus converting the endometrium to secretory and causing complete shedding of the endometrium. It is wise to warn the patient of possible heavier bleeding. Frequently one treatment is all that is necessary but if there is no bleeding in 30 days, repeat the progesterone, then if there is no bleeding in four to five days, the patient may either be pregnant, have insufficient stimulation by estrogen, or will have had spontaneous ovulation and uterine bleeding will follow within two weeks. If the bleeding response is not sufficient or absent, a full cycle with estrogen and progesterone is necessary.

Minor degrees of thyroid and to a lessor extent adrenal hypofunction and hyperfunction may also mimic the picture of dysfunctional uterine bleeding and in these patients proper therapy would be to replace or suppress the glandular function with the proper hormone or drug. Careful history, physical examination, and laboratory studies should aid in making this diagnosis. However, it must be stated that the PBI and radioactive iodine uptake reflect only thyroxin production, rather than tissue utilization. We have all seen patients with "subclinical" hypothyroidism respond on small doses of thyroid (up to 2 gr. per day) with return of normal menstrual pattern and function after an empiric therapeutic trial period of four to six months.

Miscellaneous Uses of Estrogen and Progesterone

Estrogens have certain metabolic effects, some of which have been discussed. One of the principal effects is that of the retention of water and electrolytes (sodium, potassium and chloride), therefore, its use in the treatment of such conditions as premenstrual edema, toxemia of pregnancy and edema in certain types of arthritis is ineffectual and perhaps might be harmful.

Some physicians fear estrogen administration in certain types of malignancy but this carcinogenic property has as yet not been proven in humans.

Inhibition of lactation by estrogenic therapy is questioned by many authorities but does relieve some of the symptoms of breast engorgement that accompany the active stage of lactation.

Massive estrogen therapy also has a place in the treatment of endometriosis, even though this may be symptomatic therapy rather than curative. Additional experience with estrogenic therapy is needed in this disease.

The treatment of Mittelschmerz and primary dysmenorrhea with estrogens to inhibit ovulation has a limited application. This is primarily diagnostic rather than therapeutic in most cases.

Topical estrogens are invaluable in senile, atrophic, irradiation and prepuberal types of vaginitis because of the ability of the estrogens to thicken and cause cornification of the vaginal mucosa.

Use of estrogens in the treatment of threatened and habitual abortion is probably worthless.

One major abuse is to administer moderate doses or continued doses of estrogen to children when the epiphyseal lines are still open. This therapy is known to cause premature closure of the epiphysis and subsequent arrest of skeletal growth.

Since progesterone effect in general depends on prior estrogen effect, it should be obvious that progesterone administration to treat an atrophic endometrium is valueless. Progesterone also inhibits contraction of the myometrium in the laboratory but this has not proven to be of clinical value. Progesterone is of no value in threatened or habitual abortion unless laboratory studies have shown a decreasing level and then only massive doses (100 mg. each day and higher) have been of value. The number of potential abortions, in which this criteria is present, is certainly small. Recently there have been reports of the development of artificially induced pseudo-hermaphroditism in several case of threatened abortion treated with androgens or androgen-like progestational compounds (3). The severe structural and psychic trauma to baby and family should not be regarded lightly and one should be extremely cautious before resorting to this form of therapy.

The use of relaxin type hormones is still too new to judge whether their use is beneficial or not, but as more experience has been gained, initial glowing reports have been repudiated to a great extent.

Conclusions

A basic understanding of the physiological action of hormones is necessary for rational and intelligent employment of these agents. An attempt has been made to review the knowledge of hormonal action in the obstetric or gynecologic patient. Availability of more potent synthetic preparations has made employ-

ment of physiological concept more practical. Pitfalls are many and some of the methods or modes of action are still not fully understood. If these facts are kept in mind one may greatly benefit his patient by intelligent supplementation or substitution with hormonal therapy.

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RECENT DEVELOPMENTS IN THE EPIDEMIOLOGY OF PSITTACOSIS*

MORRIS POLLARD**

The public health problem of psittacosis has expanded in recent years, coincident with changes in the social and occupational habits of man (Andrews, 1957). In temperate zones, the domestication of tropical birds has augmented the channel of infection from caged birds to man in the form of virus polluted household air. While psittacosis has been found in an ever expanding portion of the world's bird population, industrialized procedures in the processing of poultry has provided additional opportunity for exposure of employees to infection. Among the most frequently recognized reservoirs of infection in man are psittacine birds and turkeys in the United States (Steele and Scruggs, 1958), ducks in Czechoslovakia (Strauss, 1956; Sery et al., 1957), and earlier, the fulmar petrels in the Faroe Islands (Haagen and Maurer, 1939). Although man is infected usually through contact with infected birds, mild infections have been reported with no known exposure to birds (Dalgaard, 1957; Eng et al., 1957). An epidemic of fulminant psittacosis in man has been described (Olson and Treuting, 1944) in which moribund patients contaminated the air being inhaled by susceptible attendants. The origin of this epidemic too is uncertain.

The dissemination pattern of psittacosis virus among turkeys is thought to be initially via the fecal-oral route; and thereafter infection results from inhalation of infected fecal aerosols (Page, 1958). The source of infection among domestic fowl, hatched and raised under isolated conditions, is not yet defined. The virus has not been found in eggs from viremic turkey hens, it has not survived on the shell of eggs, nor on infective swabs stored in commercial incubators (Davis et al., 1957). Infected feral birds have been considered as possible sources of infection for domestic fowl; however, the evidence is only presumptive.

Psittacosis virus is a member of the psittacosis-lymphogranuloma venereum (Psitt-LGV) family whose ramifications in the animal kingdom appear to be more and more universal. The characteristics of this group have been thoroughly described by

Wenner (1958). The epidemiology of psittacosis in birds and in man has been presented in the proceedings of two symposia (Beaudette, 1955; 1958), and in a review by Schmidtke (1957). While the clinical manifestations are protean, membership in this family is based on a morphological similarity of the cytoplasmic inclusion bodies and on a common heat stable complement-fixing antigen in extracts of infected tissues.

A diagnosis of psittacosis in man depends on (1) history of exposure to birds, (2) isolation of virus from sputum, blood, or lungs, and (3) rise in serum complement-fixing titre with convalescence. Modern antibiotic therapy, however, may interfere with (2) and (3) (Pollard et al., 1953; Meyer and Eddie, 1956). Since healthy-appearing birds can be carriers of the virus, the diagnosis of psittacosis in birds depends on the pathogenicity of their tissues (spleen, liver, and kidney emulsions in saline) for mice or for developing chick embryos. The intracerebral route of inoculation in mice is the most suitable procedure for primary isolation of virus. By this route the virus induces a meningitis and the agent can be observed microscopically in smears of the meninges. A recent comparison of virus isolation by *intraperitoneal* inoculations of mice and by egg inoculations shows that the latter was more sensitive (Strauch and Rott, 1958). Pericarditis has been observed frequently in infected turkeys (Dickinson et al., 1957; Davis, Davis and Delaplane 1958), but the only lesion uniformly diagnostic of infection is the cytoplasmic inclusion body (Lillie, 1930; Coles, 1930; Levinthal, 1935) found in exudates of infected tissues. Spleen size is only of suggestive diagnostic value, since virus has been recovered from small spleens, and enlarged spleens may contain a bacterial agent.

Infection by members of the psittacosis-LGV family of viruses does not appear to induce a solid immunity with convalescence. The infective agent becomes a quiescent resident of the reticular tissue when the clinical symptoms subside. When the host is subjected to "stress," the virus may reappear and spread. Within this family of viruses immunization has been partially successful only with enzootic abortion of ewes in which abortion could be prevented in spite of a persisting infection. Exploratory studies indicate that immunization against psittacosis in birds may soon be developed. Since infected turkeys eventually eliminate the virus from tissues (Page, 1958) an immunity may be mobilized

through viral preparations incorporated in an oil adjuvant. The serological response to psittacosis infection in birds is difficult to evaluate. An indirect complement-fixation (CF) test has been used extensively and has been regarded as a very sensitive, though complex, method for detection of infection in domestic fowl (Karrer, et al., 1950). Recently, a more simple direct CF test was described for detecting antibody in turkey serum (Brumfield and Pomeroy, 1957). In all such serological tests a positive reaction indicates only that the bird had been exposed to the virus. A negative test cannot be related to infection since virus has been recovered from negative birds. In addition to the direct and indirect CF tests, a detergent-extracted skin test antigen has been used as an epidemiologic tool for detection of infected flocks of turkeys and chickens (Benedict, 1957). There is great need for a specific complement-fixing antigen by which members of this family of agents can be differentiated. Recent improvements in the Barwell (1952) technique for preparing such an antigen may provide it in significant amounts (Ross and Gogolak, 1957).

The incidence of clinical psittacosis in man is related to his exposure to infected birds. A virus-free bird offers no such hazard to a human contact. Since birds have not yet been immunized against psittacosis, an alternate more promising procedure has been explored extensively. The tetracycline compounds have definite therapeutic value for psittacosis infection in man (Cox, 1954). The treatment of psittacine birds with repeated inoculations of aqueous Aureomycin had yielded indecisive results (Quan et al., 1950); however, later attempts with twice-daily injections of chlortetracycline for 14–25 days yielded better results (Meyer and Eddie, 1955). Two procedures have now been developed which have shown application to this problem:

1. Chlortetracycline HCl has been incorporated into millet seed (0.5 mg. per gram of seed). When psittacosis-infected flocks of parakeets *melopsittacus undulatus* consumed this preparation over a period of 14 days, they were predominantly cleared of the virus (Meyer et al., 1958). By alternating the schedule of therapy (feeding medicated seed for 3 days then untreated seed for one or two days, for a total of 15–18 days) conditions were avoided wherein some of the birds developed gastroenteritis. Recent observations show that healthy-appearing birds can be fed

continuously on medicated seed—0.5 mg. per gram seed—for 21 days without harmful effect (Pollard, 1958 unpublished data).

2. An emulsion of chlortetracycline HCl in sesame oil[1] (50 mg. per cc.), injected into the pectoral muscles of naturally infected parakeets, effectively cleared such birds of detectable virus (Bussell and Pollard, 1958). Two injections of this preparation (each containing 5 mg. of the drug), two days apart into opposite muscles, has cleared the virus from 190 randomly selected birds (Table I) whereas psittacosis virus was recovered from 86 of 151 (56.9%) of the untreated control birds.

TABLE I

Composite of Four Chemotherapeutic Trials of Psittacosis-Infected Parakeets

Chlortetracycline HCL*	Number Positive for Psittacosis	Number of Birds Tested	Percent Positive
Untreated	86	151	56.9
Treated	0	190	0

* 5 mg. chlortetracycline in sesame oil into opposite pectoral muscles 2 days apart.

Recent trials show that one injection of chlortetracycline HCl (20 mg.) in sesame oil clears the virus from naturally-infected flocks of parakeets (Pollard, 1959). Table II shows a composite of

TABLE II

Composite of Two Trials:

*Treatment of Psittacosis-Infected Parakeets with Chlortetracycline in Sesame Oil**

Flock No.	Number Positive for Psittacosis	Number of Birds Tested	Percent Positive
I Untreated	10	21	46.7
Treated	0	34	0
II Untreated	15	22	60
Treated	0	17	0

* One injection of 20 mg. CTC, half into each pectoral muscle.

two such trials. Psittacosis virus was not recovered from any of 51 birds which were treated with 20 mg. of the drug. Examinations for virus were made at 25 to 79 days after treatment and

this involved three "blind" passages of test organ emulsions in mice. Virus was recovered from 10/21 (46.7%) and 15/22 (60%) of the untreated control birds.

Treatment of psittacosis-infected parrots (*Amazona oratrix*) with chlortetracycline in sesame oil (2 doses each containing 166 mg. per kilogram body weight) has cleared such birds of the virus (Pollard, 1959). Table III shows the results in three trials in

TABLE III

*Treatment of Psittacosis-Infected Flocks of Parrots with Chlortetracycline HCL in Sesame Oil**

Flock No.	Days After Treatment	Virus Isolation	
I	69–79	0/21†	(0%)
	Untreated Controls	12/24	(50%)
II	25–78	0/42	(0%)
	Untreated Controls	4/10	(40%)
III	18–52	0/30	(0%)
	Untreated Controls	11/20	(55%)

* 166 mg./Kilogram body weight, intramuscularly, two injections two days apart.
† Number of birds positive/number of birds tested.

which 93 naturally infected birds were cleared of virus, whereas virus was recovered from 27 of 54 (50%) of the untreated control birds.

While the therapeutic measures described above have freed flocks of parakeets and parrots from psittacosis infection, the results with infected turkeys have not been so definitive. It has been recommended that the administration of ground feed containing 400 grams chlortetracycline per ton will effectively clear the virus from infected turkey poults (Davis and Delaplane, 1958). This procedure has been applied in several outbreaks and the appearance of the birds has improved dramatically. An exemplary outbreak was reported among 10,200 turkeys in Oregon (Osgood et al., 1956), in which 33 confirmed and 54 suspected infections occurred among exposed personnel. When surviving birds were processed after chlortetracycline-feed therapy, heart lesions were observed in many of them. Although the virus level was suppressed sufficiently to protect personnel engaged in processing them, relapses have subsequently occurred in treated birds which had been retained for breeding stock. In this respect, the source of the returned infection has been presumed to come from an infected feral bird.

With regard to turkeys, further studies should be directed at the fate of chlortetracycline in the intestine; and the efficacy of the oral treatment should be compared with the injection method particularly as applied to breeding stock. Parenthetically, virus has been recovered from frozen dressed turkey carcasses, but the hazard to the consumer of this heat-labile agent appears to be negligible (Page, 1958).

It should be pointed out that treated birds may still be susceptible if subsequently exposed to infection. It should be pointed out also that while antibiotic-resistant mutant strains of virus have been produced through laboratory manipulations (Gordon et al., 1957), no such strain has yet been encountered in laboratory or in field-treated birds.

Through application of the procedures outlined above, the incidence of psittacosis infection in man should be controlled satisfactorily. While public health practice advocates immunization as the preferable procedure, a chemotherapeutic cleansing process can be effective if adequately applied. The treatment of birds which come in close contact with man, whether in the home or in the packing plant, should reduce the hazard of infection with psittacosis virus in man.

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PROCEEDINGS OF THE TEXAS BRANCH OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

ABSTRACTS

Of Some of the Papers Read at the Fall Meeting,

Nov. 14–15, 1958

Texas A & M College, College Station, Texas

Tissue Culture Studies of Mouse Hepatitis Virus. Theodore J. Starr and Morris Pollard, The University of Texas Medical Branch, Department of Preventive Medicine and Public Health, Virus Research Laboratory, Galveston, Texas.

The Gledhill strain of mouse hepatitis virus (MHV 1) was passaged through 10 successive tissue cultures of newborn mouse kidney explants. As MHV 1 did not cause reproducible cytopathogenic effects in our tissue culture preparations, infectivity tests with newborn mice were used to detect propagation of this virus in serially passaged cultures. Observations suggest that virus was adsorbed by the proliferating explants and that cell-free culture fluids were infective. Total time of virus growth far exceeded decay rate of the virus incubated without tissue. After the tenth passage, the virus had been diluted 10^{12}-fold which exceeded the extinction point of the original inoculum.

Influence of Environmental Temperature on Pathogenesis of Rabies in Insectivorous Bats. S. Edward Sulkin, Rae Allen, Philip H. Krutzsch, and Craig Wallis, Department of Microbiology, The University of Texas Southwestern Medical School, Dallas.

Studies on the progression of rabies virus in experimentally infected insectivorous bats maintained under different environmental circumstances suggest that the interscapular brown fat plays a significant role in the pathogenesis of rabies in this animal. The experiments included *Myotis lucifugus*, a true hibernator, and the Mexican free-tailed bat, *Tadarida mexicana*, a quasi hibernator. The study supports the proposition that the brown fat which provides food reserve during the inactive state (hiber-

nation) may also provide means for storage of the virus during the period of latency. Environmental temperature has a profound effect on the progress of experimental rabies in bats. Both the virus and the host remained quiescent when maintained in a cold environment (11°C) and both are activated when the animal is transferred to a warm environment (29°C).

Effect of Heparin on Neomycin, R. D. Higginbotham, Department of Microbiology, The University of Texas Medical Branch, Galveston, Texas.

Tissue mast cells store heparin and release this mucopolysaccharide in response to injurious stimuli. This may be an important factor in natural resistance (NYAS, 73:186, 1958). Heparin will protect mice against lethal doses of various microbial metabolites (e.g., polymyxin B, neomycin, dihydrostreptomycin, etc.) by forming acid-base complexes with them. The *in vivo* effect of heparin on persistence of neomycin antibiotic activity in mice was measured in mice challenged i.p. with a lethal dose of *M. pyogenes* var *aureus*. Neomycin (i.v., 20 ug/gm:1 LD_{10}) was slightly more effective alone than in complex with 25 ug heparin/gm. However, this heparin dose protected mice against 40 ug Neomycin/gm (1.6 LD_{100}) without abolishing the equivalent amount of additional antibiotic activity. This increased therapeutic ratio of Neomycin-heparinate in staphylococci-infected mice was discussed as a cation exchange function of heparin in the tissues.

Some Electron Microscopic Observations on Virulent and Avirulent Bacillus anthracis. I. L. Roth, C. W. Lewis*, K. C. Chao and R. P. Williams, Baylor University College of Medicine and M. D. Anderson Hospital* and Tumor Institute, Houston, Texas.

For the purpose of electron microscopic observations virulent and avirulent anthrax bacilli were each grown in a carbon dioxide system (bicarbonate nutrient agar; 50% CO_2 atmosphere) and in an air system (nutrient agar; air).

Specimens for microscopy were prepared by two methods. One consisted of suspending colonial material in water and placing a drop of the suspension directly on a screen for observation. The other method consisted of suspending several colonies in water and centrifuging to a pellet. This process was repeated twice be-

fore the final suspension was made and a drop placed on a screen.

From the electron microscopic observations it appears that the capsule of the virulent strain when grown in CO_2 is a well defined structure in contrast with the more poorly defined capsule of the virulent strain grown in air and the avirulent grown under both conditions. It appears that the avirulent organisms are capsulated, but that the capsulation is of a type different from that of the virulent in CO_2.

Phage Typing and Antibiotic Sensitivity Patterns. W. Fahlberg, E. Yow and J. Felder.

Staphylococcal epidemic phage types 44A, 80/81 and 44A/80/81 were found to be carried in the nasopharynx of the general population at almost the same incidence level as in personnel and patients in a large hospital where an epidemic of these types had just subsided. Significant numbers of *Staphyloccus albus*, coagulase-positive, were phage typed into the three groups previously described. Antibiotic resistance patterns generally reflected the spectrum of antibiotics which had been in widespread usage within the preceding six months.

Observations on the So-Called "Burn Endotoxin." C. D. Graber, A. A. Contreras and R. E. Brame.

White rats were burned to full thickness by immersion (96°/15 sec) on the dorsal aspect of a skin flap made by a modification of Selye's pneumoderma technic employed to collect a crude diffusate taken up in distilled water.

This material proved neither toxic for rats or mice, nor was there any evidence of degeneration of tissue cells of the Henle's lung or Earle's adult skin lines when perfused in a Rose chamber. There existed very little difference in the carbohydrate protein and fat moieties of burn and non-burn diffusates as determined by anthrone, micro Kjeldahl and Sudan black technics respectively. Electrophoretically the burned diffusate lacked the $\alpha 1$ fraction while the $\alpha 2$ fraction was decreased by half as compared with normal tissue. Concentration of peptides and amino acids was only slightly increased in the burn tissue as determined by paper chromotography but only to the extent that these materials are increased in burn sera. Burn diffusate in several concentrations (100 mgms-100 mg/ml) failed to elicit a positive Shwartzman in rabbits. Immunization of rabbits with crude diffusate

utilizing initially the intramuscular route with Freund's followed by I. V. injection yielded no homotypic antibody as determined by indirect hemagglutination and precipitin testing.

Recently subcutaneous air insufflation has been performed subsequent to scalding so that the muscle is involved. This method has been productive of a crude diffusate which is toxic for mice and apparently antigenic.

Pigment Production by Bacillus anthracis. Kwei-chao Chao and Robert P. Williams, Baylor University College of Medicine, Houston, Texas.

Production of a reddish-brown, pH-sensitive, extracellular pigment appeared to be a common characteristic of *Bacillus anthracis* when grown in a closed system containing bicarbonate. Of eighteen capsulated strains and two non-capsulated variants, 75% produced the characteristic pigment, while none of eighteen *Bacillus cereus* strains pigmented.

Unsuccessful attempts were made to purify the pigment. Neither organic solvent extraction nor elution from activated charcoal were successful. The pigment changed color reversibly to yellow below pH 6. As the pH was increased, the color intensity changed. Pigmented culture filtrates at pH 7.5 had absorption spectra showing either a maximum around 490 and a minimum around 450, or a plateau ranging from 450 to 500 mμ. The pigment concentration in culture filtrates was proportional to optical density.

The presence in the medium of yeast extract and bicarbonate together with a high oxygen tension was found to be required for maximum pigment production in the closed system.

Growth of Staphylococci and Production of Coagulase. J. Marston and W. J. Fahlberg, Department of Microbiology, Baylor University, College of Medicine, Houston.

Three strains of Staphylococcus aureus, 44A, 104 and the Newman strain were chosen for study of their growth characteristics and their ability to produce coagulase in brain-heart infusion dialysate and in a synthetic medium.

Characteristic growth curves correlated with coagulase activity titers were obtained and slight differences noted with the Newman strain. The relatively constant ability of B H I dialysate to support growth and coagulase production has led to an attempt at isolation of the factor or factors responsible in this medium.

A synthetic medium has been devised which allows good growth of Staphylococci and in some instances adequate coagulase production. Conditions for coagulase production will be discussed.

The Relation of Temperature and Moisture to the Survival and Multiplication of Selected Bacteria Subjected to a Simulated Martian Environment. Irving Davis,* C. E. Craft, and J. D. Fulton, Department of Microbiology-Cellular Biology, School of Aviation Medicine, USAF, Randolph Air Force Base, Texas.

Three Gram-positive soil species, a vegetative bacillus, a spore-forming bacillus, and a coccus were each inoculated into separate sterile, dry soil and subjected to simulated Martian environmental conditions. Bacteria-soil specimens periodically were analyzed for total cell counts and moisture content over 3–4 months. Preliminary results suggest that (1) soil bacteria, selectively adapted to a simulated Martian environment, survive and multiply, (2) multiplication rate of spore-formers is greater than that of strictly vegetative cells, (3) when bacteria were subjected to daily cycling temperatures (+25° to −25° C.) total cell counts appeared consistently higher than when the cells were left at room temperatures, and (4) bacteria-soil moisture content under conditions of temperature cycling appeared consistently less than moisture in samples subjected to continuous room temperature.

Growth Studies on the Cotton Root-Rot Fungus, Phymatotrichum Omnivorum. *II. The Effect of Micro-Organic Amendments.* Ralph J. Hervey, Texas Agricultural Experiment Station, Temple, Texas.

Phymatotrichum omnivorum makes fair growth on simple, mineral-carbohydrate, synthetic media. To accelerate growth seventeen recognized organic growth-promoters (plant hormones, vitamins, antibiotics) were added singly, in traces to basic media containing: NO_3 and NH_4–N, PO_4–P, SO_4–S, K, Mg, Fe, Mn, Zn and sucrose.

Experiments were conducted in shallow, stagnant liquid or on agar. Quantitative growth was determined by colony diameter the oven-dry weight.

Penicillin, gibberellic acid and cold-sterilized steer-manure extract stimulated growth most regularly in two fungus strains; carrot juice and autoclaved steer-manure extract were generally toxic. Responses varied widely with strain and nature of medium. With strain Po–2 in liquid medium streptomycin, thiolutin, bacitracin, phenazine dicarboxylic acid, terramycin and actidione (1–50 p.p.m.) inhibited or had no effect. Penicillin (10–25 p.p.m.), gibberellic acid (50 p.p.m.), 2, 4–D (100 p.p.m.), 2, 4–dichlorophenylsuccinimide (50–100 p.p.m.), chlorophenylsuccinimide (25–100 p.p.m.) and cold-sterilized steer-manure extract were stimulatory. Ascorbic acid, inositol and a chelate were toxic.

Strain Pc–5–2 was stimulated by terramycin, streptomycin and penicillin (5–50 p.p.m.), gibberellic acid and chlorophenylsuccinimide but not by 2, 4–dichlorophenylsuccinimide and 2, 4–D. On non-synthetic agar growth of poorly-growing strain Po–2 was markedly enhanced by gibberellic acid at pH 4.5 and penicillin at pH 6.5.

On synthetic agar (pH 4.5) cold-sterilized steer-manure extract stimulated Po–2 and Po–5–2; at pH 6.5 both strains were mildly stimulated by steer-manure extract, 2, 4–D, chlorophenylsuccinimide, gibberellic acid and penicillin.

The Relationship between Several Macromolecular Cell Constituents and Radiosensitivity in Escherichia coli. Daniel Billen. The University of Texas M. D. Anderson Hospital and Tumor Institute, Department of Biology, Section of Microbiology, Houston, Texas.

Through addition or omission of several chemicals in the growth medium the cellular content of ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and protein (P) can be altered both quantitatively and qualitatively. In this work the influence of such altered constituents on X-ray sensitivity of *Escherichia coli* strains B/r and 15_T^- (thymineless) has been studied. Under the influence of chloramphenicol such cells contained increased amounts of RNA and DNA relative to that of P. When log phase *E. coli* were grown in the presence of chloramphenicol and then exposed to X-rays the number of colony forming survivors was increased. An increased radiosensitivity was found when strain

15_{T-} were grown in the absence of thymine. Such cells synthesized P and RNA in abundance while DNA synthesis was essentially nil. Chloramphenicol did not enhance the radio-resistance of such cells grown in the absence of exogenous thymine. Cells grown under such stress continue to synthesize RNA only. Thus it appears that "surpulus" DNA accumulation is necessary for chloramphenicol induced resistance.

AN IMPROVED METHOD OF BREEDING GOLDEN HAMSTERS

F. J. Cusick and H. Cole*

Experiments to improve the method of breeding golden hamsters were undertaken in the hope of (1) increasing litter production; (2) decreasing the number of males injured by females; (3) reducing the time required of breeding supervisors. Since hamsters do not mate during daylight hours, and since the heat period of females could not be determined in advance, the method previously followed in the colony required that much of the operation be conducted after the regular work day. The procedure called for individual test mating so that unresponsive females and those that attacked males could be removed from the breeding cages. Responsive females were left with males until the following morning.

Development of Methods

The first problem was determination of optimum mating time. For about a month the vaginal smear method described by Kent and Mixner (1) and by Kent and Smith (2) was tried. To determine the readiness of females for mating before placing them in breeding cages, vaginal smears were examined microscopically. Ten females were selected each afternoon and left overnight with males. Successful breeding was determined the next morning by microscopic examination of vaginal content for the presence of sperm. This method resulted in the successful breeding of from 50% to 90% (an average of 70%) of the females, an improvement over the old method, and also eliminated injuries to males, and most of the overtime labor charges, but it was too time-consuming and experimentation was continued.

While checking microscopically for the presence of sperm, it was consistently observed that most of the females were shedding numerous elongated spindle-shaped squamous cells (pictured by Kent and Smith), and that when slight pressure was exerted on the lower abdomen, these females produced a heavy white sticky vaginal discharge. It was concluded that these females had com-

pleted the 4-day heat cycle. Using this vaginal discharge as an indication of readiness for breeding 3 days later, a few females were selected and all were bred successfully. Similar experiments with small groups of hamsters subsequently proved very successful.

This method was then applied to a larger group of 44 females. They were selected one morning and placed with males at 4:30 p.m. three days later. A microscopic check for sperm the following morning showed that 42 of these females had been bred successfully. After several additional weeks of experimentation with similarly successful results, the method (choosing young females three days after vaginal examination showed metestrus) was adopted for regular use in the breeding colony. The supervisor of breeding operations obtained similar results after about half an hour of instruction in the new method, and thereafter continued to do so.

The first few months of experience with the new method resulted in more non-fertile matings than had been anticipated, but apparently these were attributable to the use of too old females. As younger breeding stock became available, results improved (See Figure 1).

A serious seasonal decline was experienced in breeding from September through January of our first year with the new method. Similar declines had been experienced with our previous method, and had been reported by other breeders, to such an extent in some cases that breeding had been suspended during the winter.

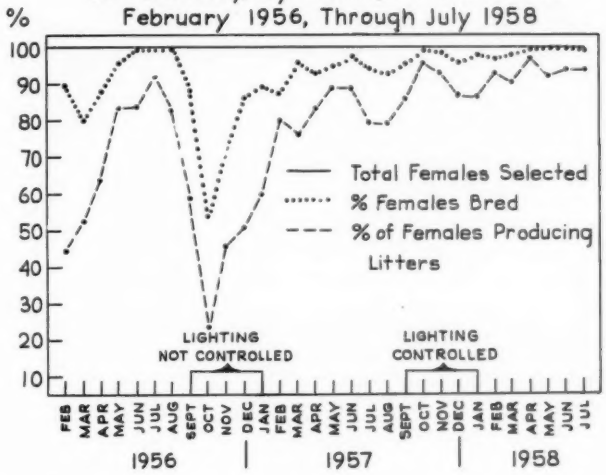
To modify or, preferably, to eliminate this seasonal decline, attention was directed to artificial illumination. The breeding colony, housed in a room with several large windows facing east, previously had been illuminated by fluorescent lights only between the hours of 8 a.m. and 5 p.m. on weekdays and for 3 to 4 morning hours on weekends. Since August 15, 1957, illumination has been provided 7 days a week from 8 a.m. to 8 p.m. and no seasonal decline has occurred.

Results

The 30-month record of the breeding colony experience, during which 8,912 females were selected for breeding, shows the effectiveness of the present method in increasing litter produc-

Fig. 1

Golden Hamsters Bred and Producing Litters, as Percentages of Total Number of Females Selected for Breeding, by Month, for the Period February 1956, Through July 1958



tion (See Figure 1). During the first year that the new selection method was followed, litter production averaged 57%, ranging from an October low of 24% to a July high of 92%. Retirement of older females further improved results. Since the new controlled lighting schedule was instituted in August, 1957, litter production has been consistently high throughout the year. In the second year, with both the new selection method and controlled lighting, litter production averaged 85%, ranging from a March low of 76% to an October high of 96%. It is interesting that before lighting was controlled litter production was lowest in October, while afterwards it was highest in that month. In the 6-month period, February-July 1958, virtually all females selected were bred successfully, and litter production varied only between 91% in February and 98% in April, averaging 94%.

In addition to the great improvement in breeding results, the new method eliminated injuries to males by females and the need for overtime supervision of breeding operations.

Conclusions

Golden hamsters have been bred very successfully, using a simple method of selecting females for breeding 3 days before mating, on the basis of vaginal discharge. This method increased litter production, eliminated injuries of males by females, and obviated the need for overtime work by personnel concerned with breeding operations. Seasonal decline in litter production was eliminated by extending the period of fluorescent illumination during fall and winter months. Retirement of over-age females from breeding also improved results.

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THE EFFECT OF EDTA ON FIBRIN POLYMERIZATION[a, b]

SHELDON F. GOTTLIEB, D. R. CELANDER AND M. M. GUEST

In 1942 Dyckerhoff, Marx and Ludwig (1) described the use of EDTA (ethylenediaminetetraacetic acid) as an anticoagulant and concluded that it acts as an antithrombin. More recently Rosenfeld and Janszky (8) suggested that the prolongation of the prothrombin time was due to the decalcifying action of EDTA. They also stated that EDTA has an inhibitory action on the formation of fibrin from a purified fibrinogen preparation. In 1955 Triantaphyllopoulos, Quick and Greenwalt (9) reported that EDTA does not have a direct effect on prothrombin or thrombin, but that its action is inhibitory to the labile factor.

Among the several possible effects of EDTA in the sequence of clotting reactions, the chemistry of the direct inhibition of fibrin formation in a purified system appears to be particularly susceptible to a controlled study. We have, therefore, undertaken an examination of the effects of various divalent cations on the EDTA inhibition of fibrin formation in an attempt to determine the nature of the inhibition.

Methods

Bovine fibrinogen, prepared by the method of Ware *et al.* (10), was stored in 0.5 ml. aliquots at —20° C. As needed, the material was thawed and sufficient imidazole buffer, pH 7.25, (6) and water were added to adjust the clottable protein concentration to 0.2% and the salt concentration to 0.9%. The fibrinogen solution was maintained at 37° C. for the duration of the experiment.

The contents of a vial of Topical Thrombin (Parke-Davis), 5000 or more units per vial, were dissolved in 2.5 ml. of 0.9% saline and stabilized by the addition of 1.0 ml. of glycerol. This solution was stored in the refrigerator.

The stock calcium solution was prepared from anhydrous calcium chloride. The other stock salt solutions were prepared from the metal sulfates. Sodium EDTA was prepared from the free acid by adding NaOH until a pH of 7.4 was attained.

The clotting times were determined in the following manner. Imidazole buffer, EDTA, and the appropriate cation solution (see Table 1 for volume and concentration of each) were added in order to 0.2 ml. of 0.2% bovine fibrinogen in a 10 × 75 mm. rimless pyrex tube at 37° C. to give a final volume of 0.4 ml. The contents were thoroughly mixed by swirling. The mixture was

clotted by dipping a glass rod, 2 mm. in diameter, into the thrombin solution and then, with a stirring motion, into the reaction mixture. The thrombin was added at least 30 seconds or more after the EDTA and cation additions since when thrombin was added within fifteen seconds no effect on clotting time was observed. This need for a delay in thrombin addition was also noted by Rosenfeld and Janszky (8). Clotting times were checked by stop watch. The formation of a firm clot was taken as the endpoint of the reaction.

Results and Discussion

The inhibitory effect of Na EDTA on fibrin formation, shown in Table 1, could have resulted from inactivation of thrombin, alteration of the fibrinogen molecule, removal of calcium ions, interference with polymerization of the fibrin monomer or a

TABLE 1

Inhibition of Clot Formation by Na EDTA and Its Reversal by Various Cations

Tube Number	Concentration of Na EDTA in the Reaction*	Cation	Concentration of Cation in the Reaction*	Clotting Time in Seconds†
	$\times 10^{-3}$M		$\times 10^{-3}$M	
1	0		0	5
2	5		0	495
3	5	Ca	3.75	150
4	5		5.0	5
5	5		12.5	10
6	5	Cu	3.75	120
7	5		5.0	5
8	5		12.5	12
9	5	Co	3.75	180
10	5		5.0	6
11	5		12.5	9
12	5	Mg	5.0	413
13	5		12.5	178
14	5		25.0	195
15	5	Fe	5.0	600 PPT‡
16	5		12.5	5 PPT

* Tube 1 contained 0.2 ml. fibrinogen and 0.2 ml. buffer; tube 2 contained 0.2 ml. fibrinogen, 0.18 ml. buffer, 0.02 ml. 0.1 M NaEDTA.

Tubes 3–16 contained 0.2 ml. fibrinogen, 0.16 ml. buffer, 0.02 ml. 0.1M NaEDTA, and 0.02 ml. of cation: Ca (0.075M, 0.1M, or 0.25M); Cu (0.075M, 0.1M, or 0.25M); Co (0.075M, 0.1M, or 0.25M); Mg (0.1M, 0.25M, or 0.5M); Fe (0.1M or 0.25M).

† In all tubes clotting was initiated by the addition of rod thrombin.

‡ PPT = precipitate formation.

combination of two or more of these. Triantaphyllopoulos *et al.* (9) have supplied evidence that the inhibition is not due to a direct effect on thrombin and Rosenfeld and Janszky (8) have shown that the inhibition does not result from the destruction of fibrinogen.

In support of the conclusion of the last named workers we have obtained a 97.5% recovery of clottable fibrinogen which had been subjected to dialysis against EDTA, followed by dialysis against NaCl to remove the EDTA. Since our fibrinogen was prepared from oxalated blood the effect of EDTA is probably not due to chelation of calcium ions. This is further supported by an experiment in which, after prolonged exposure to EDTA under conditions of dialysis, fibrinogen and thrombin were found to react normally when freed of this reagent.

Since, from the evidence available, EDTA does not inactivate thrombin, alter the fibrinogen molecule or block the formation of fibrin through the removal of calcium ions, it appears highly probable that some phase of the conversion of fibrinogen to fibrin is involved. If we accept the explanation of Ferry (2) for fibrin formation, the interference could be either with the cleavage of fibrinogen to form the polypeptide and the fibrin monomer or with the polymerization of the fibrin monomer. Reasonable explanations appear to be either that EDTA rapidly replaces, with its negative charges, the negative charges which had been removed with the fibrinopeptide or that the EDTA anion blocks the cleavage of fibrinogen by thrombin. In either case a long range electrostatic repulsion would be maintained.

If inhibition results from negative charges supplied by the EDTA anion, neutralization of such negative charges through chelation with divalent cations could be expected to overcome the inhibition. The data summarized in Table 1 appear to substantiate this postulate.

All the cations tested with the exception of iron and magnesium reversed the inhibition. The results with iron are questionable because of the precipitation which occurred. Magnesium is not as tightly bound by EDTA as the other cations and therefore would not be expected to be as effective in reversing the inhibition. It should be noted that each cation which reversed the inhibition was required at a concentration approximately equal to that of EDTA in the system. This almost mole to mole ratio

between cation and EDTA is strongly suggestive that the ionic charge of EDTA is responsible for inhibiting fibrin formation.

The interpretation that EDTA blocks fibrin formation through its negative charge is in contrast to that of Rosenfeld and Janszky (8), who ascribe a catalytic function to calcium ions in a similar system. If calcium subserves a catalytic function, it would be a reasonable supposition that other metals effective in reversing EDTA inhibition function similarly; yet magnesium, which is relatively similar to calcium, will not replace it but dissimilar ions such as copper and cobalt, are effective replacements.

The work of Robbins (7) and Laki and Lorand (4) indicates the calcium ions play a role in the polymerization of the fibrin monomer. However from their studies it appears that calcium has a greater influence in determining the characteristics of the clot than in modifying the speed of its formation.

Something more than depression of calcium ion concentration appears to be involved in the inhibition of the first stage of coagulation by citrate, oxalate, and fluoride since the concentration of these anticoagulants must be at least 2 to 3 times the theoretical amount required to remove calcium ions if the blood is to be made incoagulable. Lovelock and Porterfield (5) have suggested that the inhibition of clot formation by citrate, fluoride and oxalate is due both to depression of calcium ion concentration and to the addition of the anionic charge. However, in whole blood as contrasted with purified systems the inhibitory effect of anions appears to be even more complex. Thus Glazko and Greenberg (3) have reported that with solutions of equivalent ionic strength the same degree of inhibition can be obtained with oxalates, ferricyanides and ferrocyanides but not with other anions.

Summary and Conclusions

In a study of the effects of Na EDTA on the conversion of fibrinogen to fibrin the data obtained are indicative of direct inhibition by the EDTA anion of clot formation rather than of an induced deficiency of a specific cation. The suggestion is made that the inhibition by the EDTA anion results either from its blocking the splitting off of the highly charged polypeptide from the fibrinogen or from its rapidly replacing the negative charge

on the fibrin monomer as it is formed, thus maintaining the long range electrostatic repulsive forces and thereby preventing polymerization.

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SEX DIFFERENCE IN MORTALITY RATE OF MICE SUBJECTED TO REPEATED ELECTRICAL SHOCKS*

C. E. Hall, O. Hall, E. Cross and A. R. Adams[a]

This laboratory has been engaged in studying differences between the response of stressed and non-stressed rats to hormonal treatment (1–4), stress being induced by the electrostressor (5), a device which administers repeated electrical shocks delivered through the cage flooring. This method produces stress without also causing direct tissue damage or loss of body fluids, disadvantages common to many of the more traumatic agencies usually employed for the purpose. Heretofore experience has been confined to rats, and the present communication is to report an unexpected finding when the method was extended to the study of mice.

Materials and Methods

Experiment 1. Strain 129 mice bred in this laboratory from stock obtained from the Jackson Memorial Laboratory were utilized. They were the litter mates of dystrophic animals which had been used in other studies (6). The series consisted of twenty-four females and thirteen males between 9 and 16 months of age, averaging 13 for females and 14 for males; and was divided into two groups. Group 1, the experimental, consisted of eighteen females and eleven males; group 2, the controls, consisted of six females and two males. Those in group 1 were subjected to the electrostressor for eight continuous hours daily, during which period they received 150 V, 900 cps shocks lasting for 0.25 sec at 2-minute intervals, and were deprived of food and water. At all other times they occupied individual plastic mouse cages in which Purina Laboratory Chow and water was supplied. The experiment was concluded on the 66th day.

Experiment 2. This experiment duplicated the first in all details with the following exceptions. The mice were of greater age, 11 to 20 months for males, average 17; and 11 to 19 months for females, average 13; the shocks remained at 150 V but were raised from 900 cps to 2000 cps, and, the purpose being to compare the sensitivity of males and females to repetitive shocks, a control untreated group was not maintained. Group 1 consisted of ten males; group 2 of nine females. The experiment was concluded on the 14th day.

Experiment 3. Experiment 2 was duplicated in design, but young albino Swiss mice were used instead of strain 129 animals. Group 1 consisted of twelve males and group 2 of twelve females. The experiment was terminated on the 15th day.

Discussion and Results

The first experiment revealed clearly that males were less able to tolerate the treatment than females. A third of the males died during the first day of treatment and all were dead by the 8th day; whereas not a single female succumbed in this period, and half of them were still alive at 65 days when the stress was discontinued, as were all of the untreated controls. The results are given in Figure 1.

In the second experiment the same difference in sensitivity between males and females was evident, although males showed longer survival with 2000 cycle current than had been observed with 900 cycle. With a single exception, all males were dead by the 14th day, on which day the first of the females also succumbed. The data are summarized in Figure 2.

The results of the third experiment were less clear-cut. Females proved less tolerant to treatment than those of strain 129 and deaths among them occurred from the first day of treat-

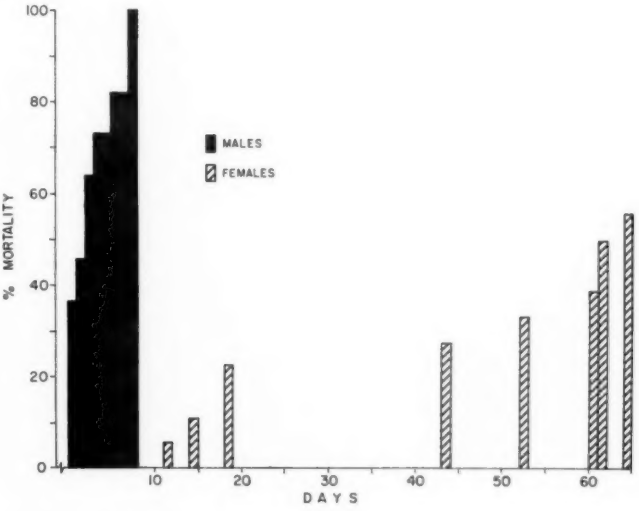

FIG. 1. Mortality rate among strain 129 mice exposed chronically to intermittent electrical shocks at 900 cps. Note that all males succumbed before the first of the females.

ment. Males died in even larger numbers throughout the course of the experiment and the mortality rate among them was virtually identical to that observed in experiment 2. This strain was much more aggressive than 129, for whereas the latter bit neither their handlers nor the flooring rods of the cage, the former, of either sex, had a tendency to do both. Five of the females and three of the males in this experiment were found dead with the teeth engaging the flooring rods, leading to the presumption that they had succumbed to fatal convulsions induced by biting these during passage of current, a finding only once encountered among mice of strain 129. The results are graphically presented in Figure 3.

In other experiments shocks have been deliberately given while mice were biting the flooring rods. Convulsions resulted sometimes, but not always, and were never fatal with a single shock. Recovery of breathing and posture was the general rule, however, in animals too stunned to disengage their incisors, successive shocks delivered in this way were sometimes fatal.

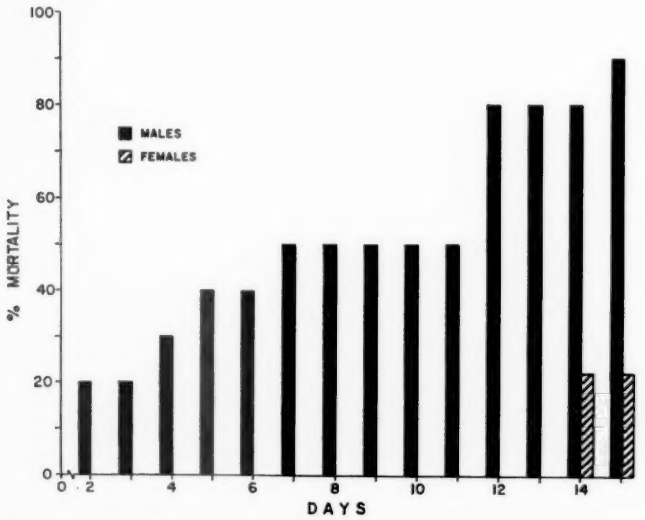

FIG. 2. Mortality among strain 129 mice exposed chronically to intermittent electrical shocks at 2000 cps. Almost all males had died before the effect began to be manifest in females.

The cause of death could not be ascertained in many of the animals, especially those which died in less than four days. Many, and this was the pattern exhibited by males in the first experiment, were observed to stiffen as the current passed and undergo a fatal seizure accompanied by cessation of respiration, a form of death to which rats given eletroconvulsive seizures are thought to succumb (7). Attempts to resuscitate such animals by resort to positive pressure insufflation of the lungs were abortive. Even though there was no evidence of fluid or other material in the respiratory passages, air could not be forced into the lungs. This, together with the fact that spontaneous respiration ceased when a convulsive seizure began, has led to the assumption that respiratory difficulty is due to either bronchiolar spasm, tonic contraction of muscles accessory to respiration or both. Others died while out of the stressing cages altogether, in their housing cages, apparently from the effects of cumulative stress. Many dying under either circumstance were found at autopsy to have

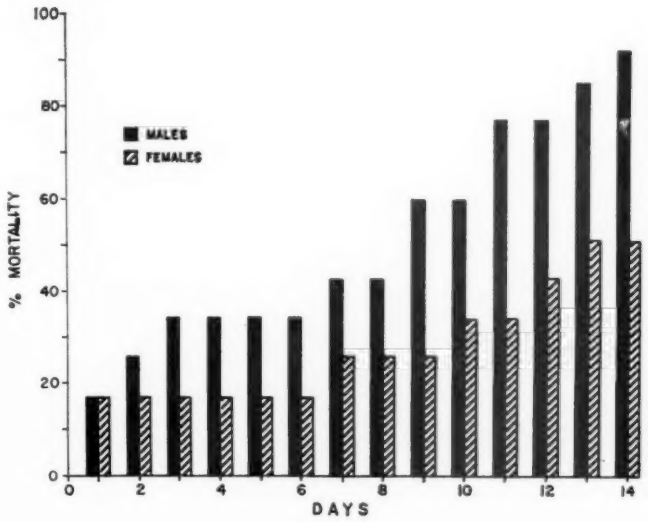

FIG. 3. Mortality of Swiss mice chronically exposed to 2000 cps shocks. Difference in sensitivity between males and females is apparent, but not as clear-cut as in the case of strain 129.

froth in the larynx and nasal passages and intense fluid congestion of the pulmonary alveoli; occasionally there was hemorrhagic lung edema. About a half dozen sustained spinal injuries, as has been reported in the rat subjected to convulsive seizures (8). Whatever the immediate cause of death, and it is evident that no single factor could be implicated in all cases, males were clearly more susceptible.

The overall results indicate that male mice are more susceptible to the lethal effects of electrical stress than females, particularly when a relatively low frequency current is used. At frequencies in the range of 2000 cps where the current takes a more superficial course, tolerance is improved. An explanation for the sex difference in sensitivity is being sought.

Summary

Male mice succumb to repeated electrical shocks sooner and in larger numbers than do similarly exposed females. The current is better tolerated at very high frequencies. Death, when the cause was ascertainable, was commonly due to pulmonary edema, although occasionally it seemed to be due to respiratory failure precipitated by a convulsive seizure.

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THE USE OF PLASTIC FOAM IN A CHAMBER FOR PAPER ELECTROPHORESIS

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The development of the chamber for paper electrophoresis described here resulted from the search for an apparatus suitable for measuring relative electrophoretic mobilities of serum proteins and sufficiently simple and inexpensive to mass produce for laboratory teaching purposes.

Two major design requirements for such an apparatus are that the paper strips be held in a horizontal position and that evaporation of the buffer solution on the paper be held to a minimum. Numerous attempts to meet these requirements have been made by sandwiching the paper strips between plates of glass and various plastics. It has usually been found difficult to prevent distortion or blurring of the pattern by such techniques due to differences in electrokinetic potentials between the plates and the paper, and to puddling which often results when the paper is removed from the plate (2).

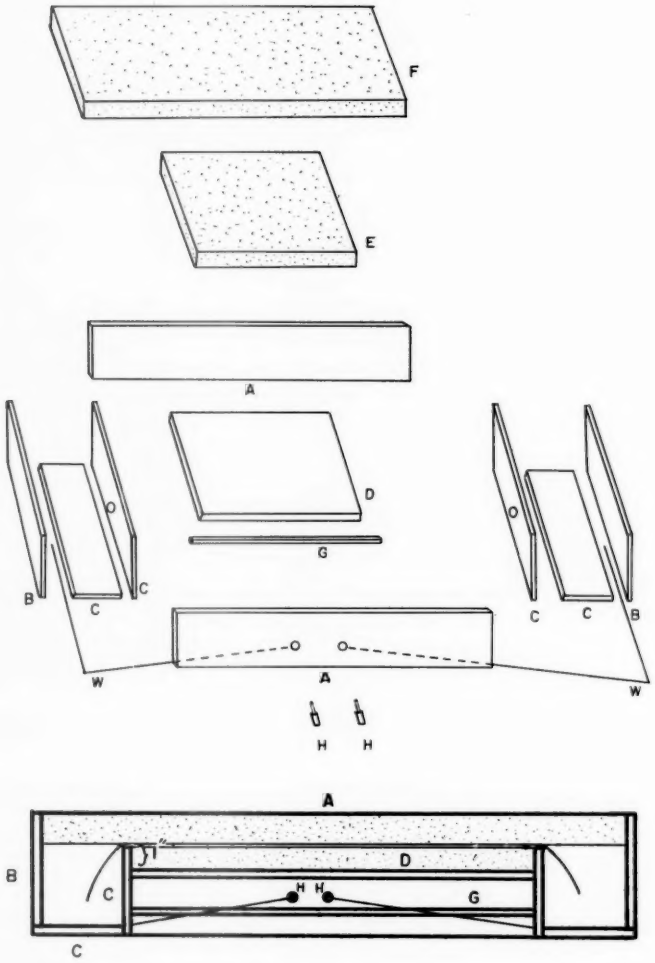
Recently, Strain (1) described the use of Styrofoam in the construction of an apparatus for continuous electrophoresis. He noted no effect on the pattern of separation of materials on a sheet of filter paper held against a block of Styrofoam. We have found, in addition, that there is no distortion of the pattern of separation of serum proteins by paper electrophoresis if the paper is sandwiched between two blocks of Styrofoam. In our chamber one block of the plastic foam serves to support the paper strips in a horizontal position and the second block serves as a lid for the chamber and rests firmly on top of the paper strips. There has been no deterioration of the plastic foam after almost a year's continuous use in the laboratory.

The dimensions of the Plexiglass pieces for the construction of one chamber are as follows:

A 2 pieces $\frac{1}{4}'' \times 3\frac{3}{4}'' \times 16\frac{1}{2}''$
B 2 pieces $\frac{1}{4}'' \times 3\frac{1}{2}'' \times 11''$
C 4 pieces $\frac{1}{4}'' \times 3'' \times 11''$
D 1 piece $\frac{1}{4}'' \times 10'' \times 11''$

These pieces are joined with a suitable cement according to the exploded view in Figure 1, and the side view in Figure 2.

Each chamber requires about 4 feet of B & S gauge number 28 platinum wire for the electrodes. The wire (W) is run along



the inside at each end of the chamber into the buffer compartments thence across the buffer compartment and along the edge formed by the bottom and outside wall. External connections to the platinum electrodes are made through binding posts (H) which are mounted on one of the 16½″ sides of the apparatus. In order to maintain the buffer solutions at the same level throughout the determination, the troughs are connected via a piece of glass capillary tubing (G) run under the 10″ × 11″ platform.

A piece of Styrofoam (E) 1″ × 10″ × 11″ is fitted snugly on the platform. This serves to support the paper strips in a horizontal position. Another piece of Styrofoam (F) 1″ × 11″ × 16½″ is used as the lid for the chamber. It should be cut to fit snugly as possible inside the chamber. In use it is pressed firmly on the paper strips. This material has enough structural rigidity to support heavy building blocks without distortion if that much pressure is desired. Both the support and lid should be brushed free of loose particles which might adhere to the paper and interfere with the staining procedure.

The simplicity of design and economy of construction of this chamber has made it advantageous for student use, and during the past year we have constructed several of these chambers for successful application in laboratory teaching.

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ANTIBODY RESPONSE TO ROUS SARCOMA VIRUS[a]

LOIS MENDLE[b] AND MORRIS POLLARD

Sera from chickens with regressing Rous sarcoma virus (RSV) tumors contain virus neutralizing antibody (Banting and Gairns, 1934; Irwin et al., 1934; Carr, 1943). Lo and Bang (1955) demonstrated neutralizing antibody in a small number of tumor-bearing chickens at 3 to 4 weeks after virus inoculation. Ligneris (1934) found that sera from fowls with rapidly growing tumors had less inhibitory power than sera of fowls with slowly growing tumors, and that sera of animals which failed to develop tumors or had retrogressing tumors were strongly inhibitory.

In early experiments on virus titration and the serum neutralization test, the experimental animal was the young chick (Bryan, 1946; Carr and Harris, 1951); however, as pointed out by Bryan (1955), there were extreme variations of biological response of chickens associated with age, a fortuitously acquired immunity, and a genetically-related susceptibility or resistance. The chorioallantoic membrane (CAM) technique has demonstrated a relationship between dilution of RSV and the number of ectodermal lesions appearing on inoculated CAMs (Keogh, 1938; Rubin, 1955, 1957; Prince, 1958). This method has now developed into a valuable serological tool in which neutralization of virus is reflected in a decisive reduction in the number of CAM lesions. Intravenously inoculated embryos appear to yield consistent reactions to RSV (Lo and Bang, 1955). This route too has been used to demonstrate a virus neutralizing effect of antibody.

In this report, the neutralization test on the CAM was employed to study the sequential changes which occur in sera of chickens during the oncogenic process. The influence of adjuvant added to the RSV inoculum on antibody response was studied in similar fashion.

Materials and Methods

For an initial serum survey, each of a group of one-month-old Leghorn chickens was inoculated intramuscularly into the pectoral region or thigh with a large dose of cell-free RSV tumor extract.[c] This inoculum was the supernatent fluid of extracted tumor homogenates, after centrifugation at

10,000 rpm for 30 minutes. When extensive fulminant tumors had developed, each bird was exsanguinated and the serum was stored at —20° C. Baseline sera had not been collected from this group of birds. In subsequent experiments, one month-old chickens were inoculated intramuscularly with purified RSV[c] (lot CT 729) emulsified in an adjuvant consisting of light mineral oil and arlacel[d] in proportions of 1:4.5:0.5 (RSV:mineral oil:arlacel). Each bird received 1 ml. of the mixture which contained approximately 4000 infectious particles as determined on the inoculated CAM. Control birds received the same amount of RSV without the adjuvant. Baseline serum was collected from each bird prior to inoculation and 3 days following appearance of a definite tumor at the point of inoculation (post inoculation serum). When the tumor was 2–3 weeks old, the bird was exsanguinated and the serum was stored at —20° C. If a bird failed to develop a tumor within 6 weeks, it was exsanguinated and the serum was similarly processed. The serum neutralization test involved 9 day embryonated eggs[e] in which the CAM was dropped by the method described by Beveridge and Burnet (1946). Inocula contained mixtures of undiluted serum, heated at 61° C. for 20 minutes, plus an equal amount of cell-free, purified virus diluted 10^{-2}, 10^{-3}, and 10^{-4}. Each egg was inoculated with 0.1 ml. of virus or 0.2 ml. of the virus-serum mixtures. Additional control inoculations consisted of 0.1 ml. amounts of saline and of heat killed virus (61° C. for 20 minutes). As a further check on the specificity of the neutralization phenomenon resulting from the virus-adjuvant inoculation, groups of eggs were inoculated on the CAM with RSV, with RSV-serum mixture, and with RSV followed by serum four hours later. Specific antiserum neutralizes the virus only when inoculated simultaneously. At least 5 eggs were used for each inoculum. After inoculation of RSV or RSV-serum mixtures, the egg shells were sealed with collodion and incubated at 36° C. for an additional 8 days. The membranes were then collected by cutting the eggs sagittally, the contents were discarded, and that half of each membrane which faced the point of inoculation was "peeled out," washed and floated in a Petri dish containing physiological saline. The number of pocks or lesions on the membrane was counted by means of a stereoscopic microscope (×10). The number of lesions on each was counted and an arithmetic average of the 5 counts was recorded to represent the average for each inoculum. Arbitrarily, a 75 per cent reduction in the CAM lesion count was selected as indication of neutralizing capacity of the test serum. The chickens used in this study were hatched from eggs supplied from the flocks at Texas A. and M. College. They were housed under isolated conditions for the duration of the experiment.

Results

Among serum specimens from 30 RSV moribund chickens of the preliminary survey group tested for RSV neutralizing properties, 17 (56%) neutralized the virus effect. Among the remaining 13 which were negative, 7 appeared to cause an RSV-enhancing effect as evidenced by CAM counts much higher and with more hemorrhagic lesions than in the controls. This enhance-

ment is not due to residual virus since the sera had been heated at virus-inactivating temperatures prior to inoculation and these sera alone were not infective on the CAM.

The effect of adjuvant on RSV antibody response is demonstrated in Table 1. Eighteen of 23 chickens inoculated with the virus-adjuvant mixture developed tumors and 17 of the latter developed neutralizing antibodies in the post inoculation sera collected 3 days after the appearance of a tumor. The other tumor-bearing chicken failed to develop RSV antibody throughout the course of the disease. Antibody was not detected in the 5 chickens which did not develop tumors.

Ten of 13 chickens inoculated with RSV alone (no adjuvant) developed tumors and one of them developed antibodies in its post inoculation serum. Serum which was collected from the latter (no adjuvant) chickens 2–3 weeks after the appearance of a tumor were tested for neutralizing antibodies and 4 out of 6 tested (66%) were positive. Three of the chickens had died before they could be bled. Three chickens inoculated with RSV alone which did not develop tumors failed to develop antibodies.

RSV antiserum, resulting from the adjuvant experiment described above, did not reduce the RSV lesion count on CAMs when the interval between virus and serum inoculation was four hours (Table 2).

Discussion

The CAM has provided a consistent technique for study of the RSV neutralization phenomenon. It should be pointed out that the range of counts within dilution levels of virus was quite wide. This required larger numbers of eggs per inoculum, and, as has been the experience of Rubin (1955) and Prince (1958), the use of more eggs has yielded more consistent results.

Serum specimens from 17 of 30 (56%) RSV moribund chickens neutralized the effect of RSV. This indicates that active infection, i.e. tumor development, may not always be associated with the production of RSV antibodies or that terminal stages of infection have interfered with the mechanism of antibody production. This might also suggest a lower threshold for induction of tumor development than of antibody response in the host. Since baseline sera were not collected from these chickens the relationship of antibody to inoculum is uncertain; antibody pro-

duction may have preceded the inoculation (Duran Reynals, 1940). It was noted that sera from 7 of these 30 chickens induced what appeared to be a virus enhancing effect on the CAM. The pock counts on membranes resulting from such serum-virus mixtures far exceeded the counts of the virus controls or the negative serum-virus mixtures, and they were often hemorrhagic. Attempts to relate this enhancing phenomenon to cortico-steroid function have thus far been negative.

The results described in Table 1 more clearly demonstrate the

TABLE 1

Effect of Adjuvant on Antibody Response to Rous Sarcoma Virus (RSV)

Chicken Inoculated With	No. of Chickens Inoculated	Presence of Tumor	Serum Neutralization Tests	
			Baseline Serum*	Post Inoculation Serum
RSV — no adjuvant	10	+	1/10†	2/10‡
RSV — no adjuvant	3	—	0/3	0/3§
RSV + adjuvant	18	+	0/18	17/18‡
RSV + adjuvant	5	—	0/5	0/5§

* Serum collected before virus inoculation.
† No. of chickens with antibody present/no. of chickens tested.
‡ Serum collected 3 days after tumor appearance.
§ Serum collected 6 weeks post inoculation.

relationship of tumor development to the production of RSV antibody. All of the antibody-producing chickens had tumors. Each of the 8 chickens which failed to develop a tumor was negative for neutralizing antibody for as long as 6 weeks after inoculation. The RSV-adjuvant inoculum induced a higher percentage (73%) of antibody-positive birds, including those negative for tumors, and an accelerated antibody response in each case. This indicates that possibly (a) prolonged exposure to the inoculated virus is necessary to stimulate antibody production, or (b) much of the inoculated virus may be inactivated or rendered non-immunogenic and the stimulus, for antibody response thereafter would depend on the reproduction of an antigenic threshold level of virus. In this respect, it appears that the adjuvant may localize the virus in the tissues long enough to promote sensitization, thereby shortening the "lag period" of antibody production. It appears from the present findings that the antibody response to RSV infection, irrespective of the lag period, is of little protective benefit in so far as tumor growth is concerned. Antibody response

of the host to inoculations with other virus-adjuvant mixtures has been studied extensively (Friedewald, 1944; Freund et al., 1948; Ward et al., 1950) and in most instances the serological pattern has been similar to that described for RSV. In our study with RSV, incorporation of tuberculosis organisms into the adjuvant-virus mixure yielded no detectable advantage in antibody response.

The antibody response to RSV-adjuvant inoculation appears to be antiviral since the addition of the same antiserum to CAMs four hours following RSV did not reduce the lesion counts below that of the virus controls. This absence of neutralization with antiviral serum after RSV has adsorbed to susceptible cells has been observed by Rubin (1955).

Summary

Rous sarcoma virus (RSV) neutralizing antibody was demonstrated 2–3 weeks after tumor development in 17 of 30 (56%) chickens moribund from the effect of RSV. Inoculation of RSV in an oily adjuvant resulted in an accelerated antibody response in a greater percentage of inoculated chickens than in the controls with virus alone. Ninety-four percent of chickens inoculated with the RSV-adjuvant mixture developed RSV antibody within 3 days after the appearance of the tumor. Within this same interval RSV antibody appeared in 11 per cent of chickens inoculated with RSV alone. It appears from the present findings that the antibody response to RSV infection, irrespective of the lag period, is of little protective benefit in so far as tumor growth is concerned.

TABLE 2

Specificity of RSV Antiserum From Chickens Inoculated With RSV + Adjuvant

Chicken Number	Lesions on CAM		
	RSV Control	RSV-Serum Mixture	RSV + Serum After 4 Hours
421 (Tumor present)	60	2	49
413 (Tumor present)	..	0	58
423 (No tumor)	..	58	82

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ULTRAVIOLET MOTION PICTURE OBSERVATIONS OF MITOSIS IN THE PRESENCE OF RIBONUCLEASE*

P. O'B. MONTGOMERY AND W. A. BONNER

The technic of time-lapse motion picture photography by means of the ultraviolet flying spot television microscope has been described (4, 5, 6, 7, 8). With this technic it is possible to obtain time-lapse motion pictures of a variety of undamaged living cells as they appear when illuminated with ultraviolet light at 2600 A°. The motion pictures thus obtained represent in the main the nucleic acid absorption images of the living cells. One of the principal areas of study with this technic has been the process of mitosis as exemplified by the auricular endothelial cell of the newt. These studies have been reported. This report records observations on mitosis in the dividing newt cell in the presence of ribonuclease.

Methods

The time-lapse ultraviolet motion pictures were obtained by recording on one 16 mm. film frame the ultraviolet absorption image obtained by exposing the living cell to one complete one second scan of the ultraviolet emitting cathode ray scanner tube employed as an illuminating source with this technic. During the following one second scan the scanner tube raster was electronically blanked so that no emission of ultraviolet occurred. This one second interval was utilized to pull the next camera film frame into position. The ultraviolet absorption image of the living cell produced by the following one second sweep of the ultraviolet scanner tube was then recorded on the new film frame following which the entire cycle was repeated. In this way the living cells were exposed to the scanning spot of ultraviolet light only on alternate one second sweeps. The cells studied were the endothelial cells of the auricle of the newt, *Triturus viridescens*. These cells were chosen for the following reasons. They require 2 to 3 hours time to complete mitosis; they have large, easily visualized chromosomes; they frequently undergo complete mitosis without rounding up; and they do not require incubation at other than room temperature. These are the ideal cellular conditions for obtaining ultraviolet time-lapse motion pictures of the process of mitosis in living cells. The cells were obtained by explanting fragments of the auricle to quartz cover slips lightly coated with chicken plasma. The plasma was allowed to clot and a drop of amphibian tyrode's solution was added. The preparation was then set up as a sitting drop in a Maximow slide and kept at 27° C. At the end of

7 to 10 days, endothelial cells will have migrated away from the explant and produced lysis of the plasma film. When a mitosis was observed to be in progress, the culture was opened and 2 drops of amphibian tyrode's solution containing 0.5 mg./ml. of ribonuclease was added. The culture was then resealed and placed under the ultraviolet flying spot television microscope for observation and time-lapse motion picture photography.

One-half mg./ml. of freshly prepared ribonuclease in amphibian tyrode's solution was introduced into the cultures at 3 stages of mitosis-metaphase, anaphase and telophase. The following observations are based on approximately 1500 feet of 16 mm. time-lapse motion pictures of about 15 dividing cells.

Results

When the ribonuclease was introduced into the culture at time of metaphase, the most outstanding and frequent change was an initial clumping and fusing of the chromosomal mass into a densely absorbing body. Following this fusion the cell generally became somewhat elongated as though preparing for telophase. The chromosomal mass elongated to some extent along with the cell and the mass appeared to swell slightly and as a consequence to show less intense ultraviolet absorption. At this state of change the majority of the cells would show an occasional peripheral cytoplasmic bubble. These bubbles contained ultraviolet absorbing material and were identical to the telophase bubbles observed in untreated, essentially normal cells. After cessation of this bubbling, the cell remained unchanged for a period of 2 hours. A rare cell would display the above changes and then progress to, and on one occasion through, telophase. In the case of the cells which did not complete telophase, a fragment of the fused chromosomal mass would be extruded from the main mass and a new, markedly abnormal nucleus would appear to be reconstituted from this small chromatin mass. The main mass of chromatin would meanwhile show no evidence of nuclear reconstruction and no cytoplasmic separation ever took place. In the case of the cell which completed division, several interesting phenomena took place. After initial fusion of the chromosomal mass at metaphase, an abnormally long period of apparent inactivity occurred. This corresponded to approximately 3 times the length of the normal metaphase. This interval was not followed by a normal anaphase, but rather by a separation of the somewhat swollen chromosomal mass into 2 more or less equal halves. The individ-

ual chromosomes could not be distinguished at this time, but several chromatin containing bridges appeared between the 2 newly divided masses. All but one of these bridges was short lived. One bridge persisted entirely through a greatly prolonged cytoplasmic separation of telophase becoming an ever thinning strand of absorbing material stretching between the 2 new nuclei. In this instance the points at which the former bridges were present persisted as spikes from the new nuclear masses of chromatin. These new masses of chromatin did not reconstitute normal appearing nuclei during the period of observation and, in addition, they remained closely approximated to the cytoplasmic borders of the daughter cells. In addition to the long chromatin bridge, cytoplasmic division in this cell was prolonged to 4 or 5 times the normal time interval resulting in formation of a long, thin band of cytoplasm connecting the 2 daughter cells for a very long period.

When the ribonuclease was introduced at time of late metaphase, one cell failed to go on to normal anaphase. The chromosomal mass remained intact until after telophase bubbling had occurred, then the mass gradually separated into 2 components joined by a rather thick ultraviolet absorbing bridge. In this case further division of the chromatin mass did not occur nor did cytoplasmic division take place.

When the ribonuclease was introduced at time of anaphase, some cells successfully completed a normal appearing mitosis while others did not. In those that did not, telophase bubbling took place normally, but there was marked chromosomal lagging as the 2 masses of ultraviolet absorbing chromatin separated. These lagging chromosomes resulted in production of chromatin spikes projecting from the surface of the new nuclei. On one occasion a chromatin bridge developed and persisted for a prolonged period as an ever thinning strand until complete telophase separation occurred. The telophase stage of mitosis in this cell was greatly prolonged and cytoplasmic connection including the chromatin bridge was maintained for a long period even though the distance separating the daughter cells was considerable.

When the ribonuclease was introduced at time of telophase, the cells completed telophase but the process was prolonged. This prolonging of the telophase state resulted in lengthening the period of cytoplasmic separation. Thus, as the new daughter cells

separated from each other, they continued to be joined by a cytoplasmic mass which grew thinner as the distance between the daughter cells increased. The daughter cells would occasionally continue to be joined in this fashion even though one of them had migrated completely out of the microscopic field.

In none of our observations was there a change in ultraviolet absorption of the cytoplasm or the chromatin which could be attributed to the effects of the ribonuclease.

Discussion

Ribonuclease is an enzyme which produces intramolecular transphosphorylation followed by hydrolysis in the internucleotide bands of ribonucleic acid. This biochemical reaction prevents the staining affinity of the ribonucleic acids for the basic dyes, but does not alter the ultraviolet absorption spectrum of the enzymatically attacked ribonucleoprotein (1). This enzyme has been demonstrated to be active *in vivo* by the author and others (2). Its action during the time of mitosis has been reported by Kaufmann and Das (3), who demonstrated that ribonuclease treated onion root tips developed a variety of mitotic abnormalities which suggested a primary enzymatic action on the spindle. These abnormalities included polyploidy, lagging chromosomes and polycentric mitoses. The majority of these changes were recorded in these experiments and may perhaps be attributed to the actions of ribonuclease on the spindle. We cannot offer direct evidence for this since the spindle has never been visualized by us as an ultraviolet absorbing structure. The markedly prolonged separation of the cytoplasm, even though the daughter cells have pulled a moderate distance apart, suggests that the enzyme has modified the cytoplasm.

Summary

1. Mitosis in the newt cell in the presence of ribonuclease has been studied.

2. Such mitoses frequently show metaphase arrest, chromosomal lagging, chromosomal bridging, prolonged delay in cytoplasmic separation at telophase and abnormal nuclear reconstruction.

3. Ribonuclease does not produce ultraviolet absorption changes in living cells during mitosis.

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THE REARING OF HOMOZYGOUS HAIRLESS DEER MICE, PEROMYSCUS MANICULATUS GAMBELI, WITH A NOTE OF SUPERFETATION OR DELAYED FERTILIZATION[a]

A. A. Packchanian and J. G. Sinclair

The studies of the mechanism of hair loss and its possible prevention would be facilitated if strains of homozygous hairless mammals were available, particularly those in which hairs are present in the young but which invariably disappear a few weeks following birth, without exception, in all of the broods. The present communication pertaining to successful rearing of offspring from homozygous hairless parents of deer mice (*Peromyscus maniculatus gambeli*) makes such investigations possible.

Hairlessness in Mammals

Gordon in 1850 was the first to describe three hairless mice (*Mus musculus*). The mice destined to become hairless were born with a coat of hair; however, after two to three weeks of hair development, it began to thin out. Eventually there was practically no hair left except for that of the muzzle and a few scattered hairs on various parts of the body. The skin of older hairless mice at times became corrugated and was termed "rhino" skin.

Since the early report of Gordon, several investigators have reported hairlessness in other species of mammals, such as: deer mice, Asiatic mice, rats, rabbits, horses, cattle, goats, swine, dogs, and even in man. Most of these findings have been reviewed by Summer in 1924, by David in 1932 and by Rigdon and Packchanian in 1957.

Both albino and colored hairless deer mice (*P. m. gambeli*) were first reported by Summer in 1924. They appeared in two apparently independent descent lines. In one case, the first hairless animal was only one generation distant from one of its wild ancestors, while in others there had been a lapse of four generations. The hairlessness was a simple recessive characteristic. The animals destined to be hairless were born with a coat of hair;

however, it began to thin out after two or three weeks. Eventually there was practically no hair left except for that on the muzzle and a few scattered hairs on various parts of the body. The albino and colored strains with hair were bred without difficulty, but the homozygous female hairless was believed to carry with it a certain lethality which was described as general infertility with poor development of mammary glands, inability to produce milk, and lack of resistance to exposure. Sumner and other investigators since have maintained hairless deer mice in laboratories as heterozygous stock either with continual backcrosses of hairless male with normal female or with male and female mice with hairs, one or both of which were heterozygous for hair and hairlessness (1, 2, 5, 6, 7).

According to David's studies and review in 1932, three main genetic types of hairlessness in mice were associated with the following histology: (a) Where hairlessness was dominant, the heterozygous condition was manifested by an imperfect formation of hairs. (b) When it was homozygous for dominant hairlessness, the keratinization was imperfect and the hairs were sheared off at the surface. (c) When the hairlessness was inherited as a recessive characteristic as it is in both *Mus musculus* and *P. m. gambeli*, the hair bulb was lacking and the hair follicle became a cyst with more or less sebaceous content leading to vesicular "rhino" skin. The homozygous individuals from their birth up to the second or third week of their lives appeared normal. After this time their hairs were gradually lost and the next generation of follicles developed imperfectly. The claws, of relatively soft keratin, in hairless mice have a tendency to become long and spiraled (1, 4, 9).

Rigdon and Packchanian in 1957 described in further detail the condition of the skin and hair follicles of hairless deer mice both in young while temporarily possessing hairs and hair coats and in completely hairless young and adults.

Materials and Methods

The hairless strain of deer mice (P. m. gambeli). Breeding pairs of colored, albino and hairless deer mice (*P. m. gambeli*) from offspring of Sumner's original stocks were received through the courtesy of Dr. Lee Dice of Ann Arbor, Michigan, during 1929 and again in 1936 by one of us (A. P.). Since 1936, these strains of deer mice have been reared in the laboratories by one of us (A. P.), from 1936–1941 at the National Institute of Health, Washington,

D. C., and from 1941 up to the present time in Galveston, Texas. During 1937 at Washington, D. C., one of the hairless females of albino *P. m. gambeli* gave birth to and reared successfully a single male offspring. This individual was returned to the general stock. Up to 1956, backcrosses were used for maintaining the hairless stock from which, after careful selection and care, the present homozygous hairless stock was reared.

The rearing and care of offspring from completely hairless parents. Twenty-three pairs of completely hairless male and female deer mice (*P. m. gambeli*) were mated. Of these, 11 pairs were albinos, nine colored and three mixed albino and colored. Sixteen matings were made in wire cages and seven in jars with equal success. Their food consisted of Purina dog chow plus water daily and carrots twice a week. An adequate amount of cotton was placed in the jars or cages which served as a nest and coverage. When the female was in the late stage of pregnancy or as soon as the babies were born, the male was removed from the female, and the date of birth of litter, their number and the ability of the mother to nurse the babies were recorded. Care was taken not to disturb the mother. The young, after shedding their hairs and when about 30 days old and completely hairless, were removed and usually mated some two to three months later. In general, the handling of the mother and the litter was avoided for ten days after birth. This procedure usually reduced cannibalism of babies by the mother. The same procedure was used for all later generations. The general care and handling of deer mice throughout all of these studies were essentially the same as described by Dice (2) and by Packchanian (5, 6).

The selection, care and rearing of hairless house mice (*M. musculus*), the original stock from Bar Harbor, Me., was generally the same as described above for the deer mice (*P. m. gambeli*).

Results

Rearing of homozygous hairless deer mice (P. m. gambeli). Out of the matings of 23 pairs of hairless *P. m. gambeli* males with hairless females (11 albino, nine colored, and three mixed pairs), 13 were successful (five albino, five colored, and three mixed pairs). An additional albino pair was found fertile; however, the mother cannibalized her offspring shortly after their birth. The remaining nine matings, consisting of five albino and four colored, were unsuccessful.

The 13 fertile pairs had 24 pregnancies, three of which were delayed fertilization, or superfetation. They reared a total of 58 offspring to full maturity with an average of 2.4 individuals for each pregnancy.

After birth all the young had hair which developed into a full coat, white or colored, depending on whether the parents were albino or colored, during the first two weeks of their lives (see

the young in Fig. 1A); however, the following week the hair gradually fell out, usually first from the head (see the young in Fig. 1B), and a few days later from all over the body (see Fig. 1-C). Finally all the individuals became completely hairless (see adults in Fig. 1 A, B, C).

Some of the offspring two to three months of age or older, when mated, were found to be fertile and homozygous for hairlessness.

Rearing homozygous hairless house mice (M. musculus). Out of 44 pairs of homozygous hairless *M. musculus* (16 albino and 28 colored pairs) nine were successful (five albino and four colored pairs). Two additional pairs gave birth to litters; however, they were cannibalized by the mothers the following day. Matings of the remaining 33 pairs were unsuccessful. Of 10 pregnancies from nine pairs, one was due to delayed fertilization. A total of 37 offspring (18 albino and 19 colored) were reared by nine mothers to full maturity, an average of 3.7 individuals per pregnancy. All of the offspring had hair which developed normally to full coat within two weeks, after which the hair began gradually to disappear, and finally within a month all individuals, without exception, became hairless. Some of these offspring mated at two or three months of age or older were found fertile and homozygous for hairlessness.

Superfetation or delayed fertilization in homozygous hairless mice. Out of 24 pregnancies from 13 pairs of *P. m. gambeli*, superfetation or delayed fertilization was noted in three: (a) One of the pairs, (albino male and female) mated on 8 October, the female became pregnant in the early part of January and gave birth to a litter of two on 26 January; the male was removed at this time from the female, and the second birth of two offspring from the same mother occurred on 19 February, 24 days following the removal of the male. (b) A second pair (colored male and albino female) were mated 24 November. The female apparently became pregnant in the middle of December and gave birth to a

FIG. 1. Homozygous offspring from three hairless parents of deer mice, *Peromyscus m. gambeli*. (A) Hairless mother with three offspring 12 days old still possessing a good coat of hair (which disappeared two weeks later). (B) Another mother with two offspring 18 days old showing the loss of hairs on the head (this brood became completely hairless later). (C) A mother with 25 day old offspring, about 90% hairless (the young became completely hairless within a month).

A
B
C
1cm

brood of two on 6 January; the male was separated at this time, however, and the birth of the second brood of two from this mother occurred on 2 February, 27 days after separation of the male from the female. (c) The third superfetation was noted in another hairless pair (albino male and female) which were mated 24 November. The female became pregnant in the middle of January and gave birth to a brood of two on 7 February, at which time the male was removed. The second delivery of an unknown number of offspring from this female took place on 5 March, 26 days following the removal of the male from the female.

Out of ten successful pregnancies of homozygous hairless *M. musculus* from nine pairs, one was due to delayed fertilization. This pair was placed together 24 November. The female was noted to be pregnant on 14 January when the male was removed. The first litter of three was born on 30 January, and a second brood of three from the same mother took place on 25 February, 42 days after removal of the male from the female.

Discussion

Experimental data on hand reveals that with the careful selection and mating of homozygous hairless male and female deer mice, *Peromyscus maniculatus gambeli*, and with proper care, the hairless female not only will become pregnant, but what is more significant, she will rear the offspring to full maturity. Since the offspring during pregnancy and while they have hair during the first two weeks of their lives (white or colored hair depending on whether the parents are albino or colored) are destined invariably to become completely hairless within about a month, they would be valuable experimental animals for studies pertaining to various stages of hair development from embryonic life up to the hairless stage.

Rearing of homozygous hairless deer mice in wire cages and jars is apparently equally satisfactory. The removal of the male from the female as soon as she becomes pregnant and avoidance of disturbances of the mother while in the late stage of pregnancy and until the offspring are at least seven days old are essential. With such precautions and with constant supply of food and

water, the percentage of offspring reared to full maturity is increased and possible cannibalism of the babies by the parents is decreased. The successful rearing of homozygous hairless mice in the present studies cannot be altogether attributed to care. The early observation (Packchanian, 1937, 1956 unpublished data) that a few individuals were able to suckle from hairless mothers which were mingled with the breeding stock may have resulted in the selection of mothers capable of rearing babies and ability of the babies to survive. The mammary glands of hairless females from heterozygous mothers with hair are generally small and flat.

Delayed fertilization, or superfetation, in which the birth of a brood is delayed considerably behind the normal period of gestation and a later brood results from insemination of the mother during or before the end of pregnancy is not as rare as is commonly believed.

Since the normal period of gestation in *P. m. gambeli* is about 22 days and in *M. musculus* is about 21 days, there is no doubt that delayed fertilization did occur in four instances in homozygous hairless mice during the present investigation.

As early as 1916 Summer noted two instances of delayed fertilization in his stock of Peromyscus, once in *P. m. gambeli* and another in one *P. m. rubidus*. Eighteen years later Summer (9) reported two additional such instances in his stock of *Peromyscus*. Although the exact mechanism of delayed fertilization is unknown at the present time, among several possible explanations, Summer (9) stated three: (a) a second effective copulation, accompanied by ovulation, occurred during the first pregnancy; or (b) ova liberated during this period were fertilized by sperm held in the fallopian tubes from a previous copulation; or (c) the ova for both broods were all fertilized at the same time, but for some reason the development of the second lot was prolonged.

Delayed fertilization both in mice and rats has been observed frequently in several laboratories but most of such observations are not published. The high incidence of superfetation in homozygous hairless mice, about 13% in *P. m. gambeli*, suggests that this may occur frequently, at least in the present stock. Further studies on delayed fertilization and the exact determination of

localization of sperm in the holding process or of superfetation in the presence of implanted embryos will undoubtedly throw more light on this interesting phenomena.

Summary

1. Successful rearing of homozygous hairless American deer mice, *Peromyscus maniculatus gambeli*, was secured from 13 out of 23 pairs of hairless parents. The offspring from these matings were born with hairs which developed into a full coat within two weeks. After this period, the hairs began to fall out gradually and within a month, all the offspring, without exception, became hairless. Of 24 successful matings of 13 pairs of hairless parents, 58 offspring were reared to full maturity by their mothers, an average of 2.6 individuals per each pregnancy. The hairless offspring were found fertile and homozygous for hairlessness.

2. From 44 matings of homozygous hairless mice, *Mus musculus*, nine pairs were successful. The offspring from ten pregnancies (from nine fertile parents) were reared by their mothers to full maturity, an average of 3.7 mice per each pregnancy. The offspring when fully mature were found fertile and homozygous for hairlessness.

3. Delayed fertilizations, or superfetations, were noted for the first time in homozygous hairless mice, one out of ten pregnancies (from nine fertile parents) in homozygous hairless *M. musculus* and three out of 24 pregnancies (from 13 fertile parents) in homozygous hairless *P. m. gambeli*.

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THE SUBSTITUTION GASTRIC POUCH (A)

EDGAR J. POTH*

Total gastrectomy is being done with increasing frequency and diminishing mortality. The importance of the construction of a substitution gastric pouch has become of added importance with the greater incidence of total gastrectomy. Such pouches have been constructed from distal ileum and right colon by Lee (4) in 1951 and Hunnicutt (2) in 1952. Lee (4) suggested a segment of transverse colon be used for the same purpose. A jejunal segment was utilized to form a pouch by Hunt (3) in 1952. Moreno (5) reviewed this subject recently and gave a rather complete bibliography. Randall (7) and his co-workers in reviewing the status of these pouches came to the conclusion that none of them served as true reservoirs, but rather that food passed through them rapidly with the result that they do not fulfill their function and neither increase the patient's ability to take large meals nor reduce the tendency for the development of the post-gastrectomy syndrome, "dumping." Occasionally the postgastrectomy syndrome of "dumping" is not present even though the esophagus is sutured directly to the duodenum. Although the symptoms of "dumping" are absent, a meal observed by fluoroscopy will pass directly through the conventional pouch without significant delay. The good result obviously is not due to the presence of a functioning pouch. A substitution gastric pouch which will function as a true reservoir for food is greatly needed. The idea of introducing a reverse segment of bowel distally to act as a physiological valve was entertained, but it took some time before the scheme illustrated in the accompanying diagrams was conceived. While it seemed highly desirable to use a short reverse segment of bowel at the outlet to permit its anti-peristaltic activity to function as a retarding valve, a method by which this could be done without using a short segment of jejunum twisted on its pedicle was not visualized and delayed its being done for several years. A short segment of bowel with a narrow twisted mesenteric pedicle would, of course, introduce a very real hazard and might well preclude its clinical use. With the conception of the procedure demonstrated in the accompanying diagrams, it

became feasible to introduce a reverse segment of bowel at the outlet without the hazard of ischemia and necrosis.

Not only is this procedure applicable to total gastrectomy, but it is used in conjunction with subtotal gastrectomy or when an individual who had had a subtotal gastrectomy with Bilroth II type of procedure develops severe "dumping" not controllable by medical means.

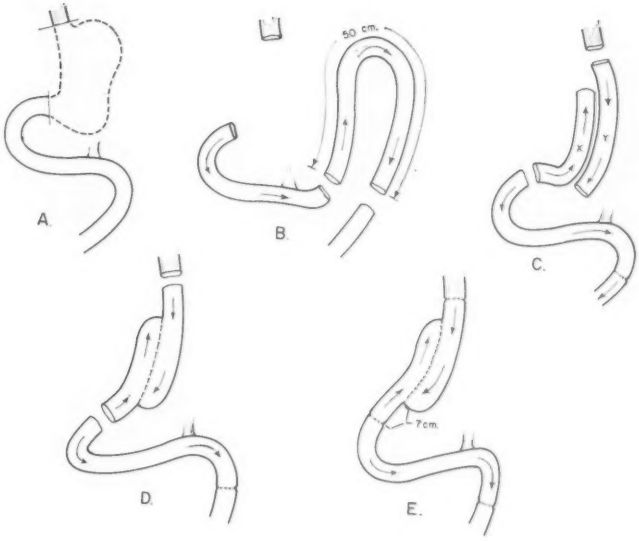

FIG. 1. Line drawings demonstrating the principal operative steps in the construction of the jejunal pouch with an antiperistaltic segment of jejunum as the outlet.

B shows the jejunal loop, with its mesentery intact insuring unimpaired blood supply, brought up retrocolic. This loop is divided in *C* with just sufficient section of the mesentery (about 4 cm.) to permit the slippage of the segments X and Y to form the pouch in *D* by longitudinal suture posteriorly and anteriorly, using two rows of continuous 2–0 catgut. The antiperistaltic segment 6 to 7 cm. long forms the outlet. The exact length of this reversed segment is not critical and can vary considerably without perceptibly changing the function and physiological valve action.

Anastomosis of an isoperistaltic segment of jejunum to the esophagus prevents regurgitation and is especially important where the outlet of the pouch is antiperistaltic.

The Surgical Procedure

This procedure divides itself into two distinct choices: one wherein the duodenum is used, which is highly desirable when possible; and the other when the duodenum must be excluded. Inclusion of the duodenum in the alimentary stream may well be of importance in stimulating increased biliary and external pancreatic secretion to aid in fat and protein digestion. The actual operative procedures require little detailed description beyond that given in the accompanying figures.

The following case reports indicate the convalescent course followed. Case No. 1 is the first patient operated upon and is approximately three years postoperative.

Case No. 1. M. D., colored female, age 67, admitted to The University of Texas Medical Branch Hospitals April 19, 1956, following which a clinical diagnosis of carcinoma of stomach was made and exploration done May 11, 1956 revealing a tumor occupying the greater portion of the stomach and originating in the mid lesser curvature. Grossly the characteristics were those of a malignant lesion. The liver was free of visible gross metastases. A total gastrectomy was done and substitution pouch constructed as indicated in Figures 1 and 2. No malignant cells were found. Convalescence was relatively smooth, maximum temperature elevation of 1.5°C. on third postoperative day. Oral feedings begun on sixth postoperative day with some regurgitation until fourteenth postoperative day. Food was taken well thereafter. Attempts to "dump" this patient with hypertonic glucose solutions were unsuccessful, even when as much as 250 gm. of glucose were given in saturated solution.

X-ray examination using a meal of food mixed with barium on the twentieth postoperative day showed the pouch to empty piecemeal and somewhat more slowly than the normal stomach. It is interesting that the first bit of food ingested passes through the pouch rapidly quite as is true with the normal stomach. A trace of barium was still present in the pouch after 24 hours. A similar examination three weeks later showed emptying of pouch to begin in about fifteen minutes with 30 per cent of barium remaining at four hours. A churning action can be seen in the substitution stomach.

There has been a slow steady gain of weight since operation. On May 21, 1956, the hemoglobin was 7.8 gm. and on Feb-

ruary 19, 1957 it had risen to 10.2 gm. Patient has received iron gluconate but no hydrochloric acid.

This patient has no complaints referable to the gastroenteric tract, eats a varied, unrestricted diet of three meals daily and goes about her usual duties unhampered.

While this patient was the first individual on whom this procedure was performed, macrocytic anemia has not developed but

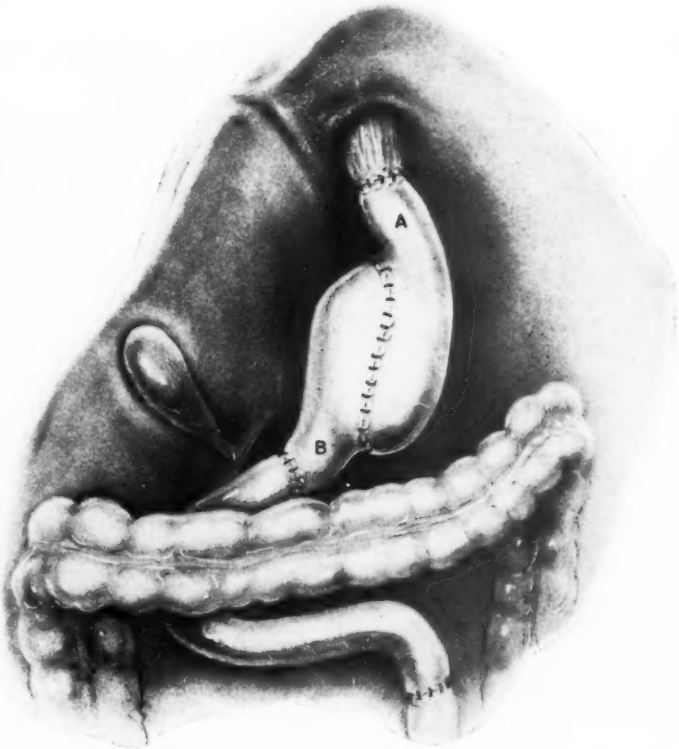

FIG. 2. The completed jejunal pouch in classic form. *A* sutured to the esophagus in isoperistaltic direction to prevent regurgitation and there has been none.

B is the antiperistaltic outlet which serves as a physiologic valve retarding emptying and permitting the pouch to evacuate piecemeal. After the immediate postoperative period, "dumping" does not occur.

there might not yet have been sufficient lapse of time.

Case No. 2. B. R., 29-year-old Latin American woman, was admitted to The University of Texas Medical Branch Hospitals

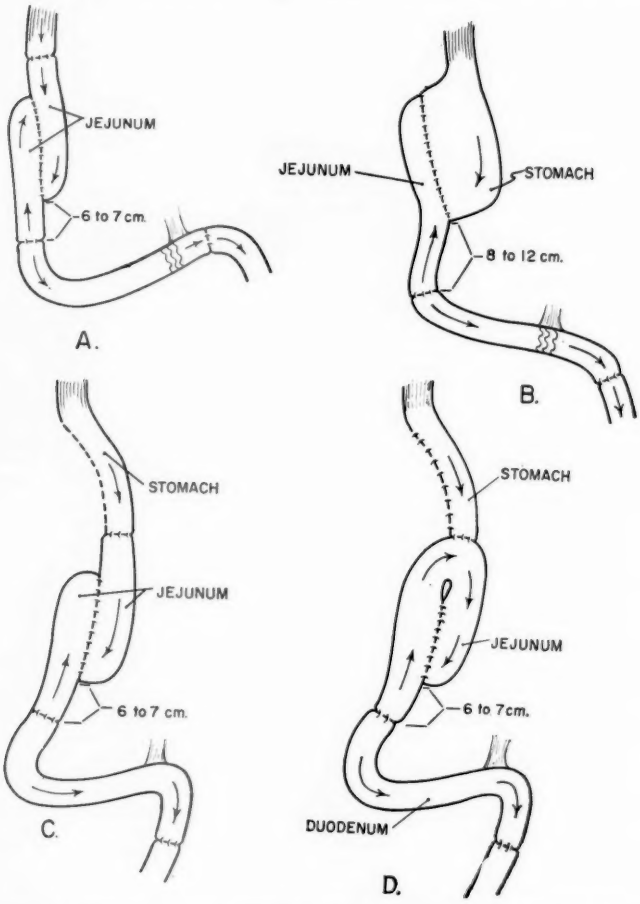

FIG. 3. These schematic drawings show continuity with the duodenum. *B*, *C* and *D* show remnants of stomach retained. The motor activity of the stomach making up part of the pouch requires a longer antiperistaltic segment to retard emptying. The arrangement in *D* is probably superior to that of *B* and *C*. *A* and *D* are likely the most desirable of the possible arrangements.

November 4, 1956. Her history is complicated with numerous hospital admissions and operations dating back to massive hematemesis in November, 1954.

First operation—December 14, 1954: Resection of two-thirds of stomach. Hemorrage again January 5, 1955.

Second Operation—January 11, 1955: Gastrotomy, left gastric artery ligation, Vagotomy.

Third Operation—July 1955: Repair hiatus hernia, esophageal ulcer present. June, 1956 stomal ulcer demonstrated.

Fourth Operation—June, 1956: Esophagogastric resection including lower 6 cm. of esophagus, transthoracic.

Continued to have a great deal of pain, was considered uncooperative and diagnosed as a malingerer by neuropsychiatric staff July, 1956.

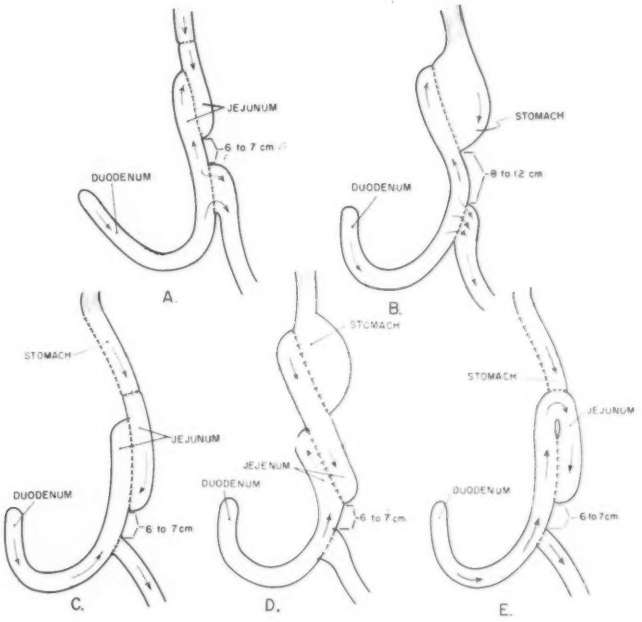

FIG. 4. Diagrams illustrating the possible arrangements when for one reason or another the duodenum cannot be included in the continuity of the enteric tract. *A* and *E* represent the most likely possibilities.

Fifth Operation—July 18, 1956: Preoperative diagnosis, repeated peptic ulcerations, massive hemorrhage, possible retained prepyloric mucosa, possible pancreatic tumor, extensive ulceration of gastric remnant, esophageal stricture incomplete. Exploration—amputation of proximal duodenum and contiguous gastric mucosa.

Patient continued to have pain in shoulder, gastrointestinal hemorrhages and esophageal stenosis.

Sixth Operation—June 25, 1957: Exploratory diagnosis—gastric ulcer invading left lobe of liver, left diaphragm and peri-

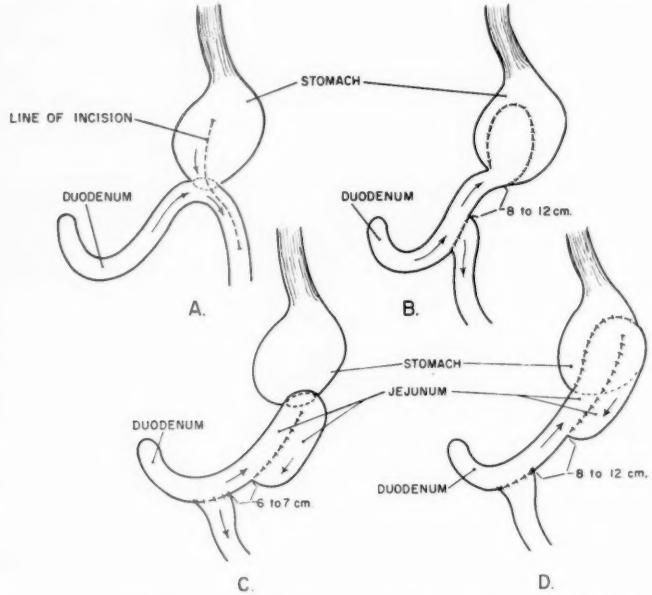

FIG. 5. Illustrating the application of the principle when it becomes desirable to control intractible "dumping" following a Bilroth II type of gastrectomy. *A* shows the line of incision through the stoma permitting the conversion sketched in *B*. The length of the antiperistaltic segment should be 8 to 12 cm., because the action of the gastric musculature will tend to empty the pouch relatively forcefully. *C* shows the formation of a double compartment and is applicable when the afferent loop is long. *D* a combination of *B* and *C*. The length of the antiperistaltic segment is dependent upon whether or not the gastric musculature can forcefully empty the pouch.

cardium, esophagogastric stenosis, extensive gastric fibrosis. Excision of gastric remnant, esophagojejunostomy isoperistaltic, jejunojejunal pouch, jejunoduodenostomy antiperistaltic and duodenojejunostomy as indicated in Figure 4A.

Received jejunal feedings from first postoperative day via jejunostomy tube. No oral fluids until ninth postoperative day. They were well tolerated. Solid food orally on fourteenth postoperative day.

Dismissed from hospital June 24, 1957. Eating well, gaining weight and strength. Weight 94 pounds.

Followup—September 3, 1957: Asymptomatic, eating well, no "dumping" symptoms, weight gain to 107 pounds (13 pounds in six weeks). Hemoglobin 11.7 gm. September 15, 1958: weight 117 pounds; hemoglobin 14.7 gm.

Case No. 3. F. D., white male, age 57, admitted to University of Texas Medical Branch Hospitals, September 25, 1956 for epigastric burning dating back to 1951, clinical diagnosis of prepyloric carcinoma of stomach made. Diagnosis confirmed at operation October 19, 1956 upon finding a prepyloric mass 6 cm. in diameter with a 2 cm. ulceration. Nodes taken from lesser omentum showed no malignant cells on frozen section.

Eighty-five per cent gastric resection and splenectomy performed followed by construction of pouch as shown in Figure 3b.

Microscopic diagnosis: Undifferentiated adenocarcinoma with metastases to the regional nodes. Uncomplicated postoperative convalesence. Gastric suction discontinued on fifth postoperative day and small quantities of fluids taken by mouth. Taking small amount of solid food on seventh postoperative day.

Taking three meals daily after dismissal from hospital without symptoms of "dumping."

X-ray examination on February 2, 1957 showed rapid emptying of pouch.

Repeat examination on April 8, 1957 shows piecemeal emptying of stomach with approximately 40 per cent retention after two hours, 20 per cent at 3 hours and ten per cent at four and six hours. Hemoglobin 14.3 gm. on September 10, 1957. Not taking iron.

Eats three regular meals and drives a tractor twelve to fourteen hours daily.

Preoperative weight 152 pounds, dismissal weight, November 11, 1956, 135 pounds. Followup weight, September 10, 1957, 132 pounds. Has no complaints and continues to do hard labor.

Discussion

What will be the fate of the antiperistaltic segment of Jejunum? Will it become dilated and functionless, will it remain unchanged or will it become greatly hypertrophied and end in obstruction? Observation up to practically three years indicates a moderate amount of hypertrophy occurs resulting in improved function of the pouch. The emptying time is prolonged, but in no instance has there been either clinical or X-ray evidence of undesirable gastric retention. Immediately after operation a pouch frequently evacuates too rapidly, but within two to three weeks partial, piecemeal emptying supervenes to give prolonged retention with simultaneous development of appetite and disappearance of all symptoms of "dumping." The incidence of 52 and 53 per cent of at least minimal symptoms of "dumping" after vagotomy and gastroenterotomy and partial gastrectomy respectively reported by Everson, Hutchings, Eisen and Witanowski (1), and the occurrence of total invalidism due to "dumping" of two and one-half per cent presented by North (6) in an unpublished report on a series of nearly 200 individuals subjected to two-thirds to three-fourths gastric resection for duodenal ulcer, indicates the importance of this complication.

Since the first portion of food ingested is passed so rapidly, it is unexpected that these pouches then empty slowly and piecemeal. It should be emphasized that the colon is not suitable for construction of a sustitution gastric pouch, because the colon normally evacuates by mass contraction. Should a segment of colon be anastomosed to the esophagus in the reversed order, it can be expected to result in troublesome esophygeal regurgitation.

Both microcytic and macrocytic anemias may follow total gastrectomy. Microcytic anemia results from failure of absorption of ferrous iron. The absence of acid in the upper gastroenteric tract favors this failure. An alkaline medium is optimal for oxidation of ferrous to ferric iron and both ferric and ferrous irons are precipitated at this pH. In either instance ferrous iron is not available and microcytic anemia results. The administration of fer-

rous iron and even small quantities of hydrochloric acid or parenterally administered iron prevent the development of this type of anemia. A small remnant of stomach might well supply sufficient acid and it might also supply the required intrinsic substance to assure the needed absorption of B_{12}.

It seems odd that the development of macrocytic anemia is delayed so long after total gastrectomy if the stomach is the sole site for synthesis of the intrinsic factor. Can there be other sites of such synthesis? Does macrocytic anemia develop after a delay of two to five years because this is the period of time required for exhaustion of another site or sites rather than for the disappearance of preformed, stored intrinsic and/or extrinsic factors? Will adequate food intake alter this course in any manner by improved nutrition? It seems unlikely that an organic molecule as complex as vitamin B_{12} would be sufficiently stable chemically as to persist stored in the liver for five or even more years.

The increased capacity of ingestion and the slowed transit of food through the intestinal tract, especially where the duodenum is in continuity with the substitution pouch, may improve digestion and absorption of protein and fat and may better nutrition.

These questions are the subject of continued study.

In contradistinction to the finding that the usual jejunal pouch with an isoperistaltic outlet functions for a limited period, a jejunal pouch with an antiperistaltic outlet improves in its function for a time then stabilizes and continues to function satisfactorily up to the limited period of observation afforded to date.

Summary

A substitution gastric pouch with an antiperistaltic outlet functioning with a valve-like action has been constructed to yield a vesicle which serves as a true food reservoir emptying slowly in a piecemeal fashion, slowing transit through the upper enteric tract and eliminating the phenomenon of "dumping." Will this gastric reservoir be adequate to improve enteric absorption of the extrinsic factor, vitamin B_{12}, sufficiently to prevent the development of macrocytic anemia?

These various operative procedures have been done to date on 27 occasions.

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STUDIES ON ADRENAL ASCORBIC ACID. V. RATE OF REPLETION OF DEPLETED GLANDS

LOTHAR L. SALOMON[a]

Experiments were described earlier, which indicated that exchange of ascorbic acid between adrenal glands and extracellular fluid is a biphasic process, resolvable into a fast and a slow step (2, 3). The steady-state rates of these steps were measured. Since the slowly-exchanging fraction alone is affected by ACTH (2), it is possible that the rate at which ascorbic acid accumulates in the depleted gland might be controlled by the same factors, which govern the steady-state exchange of that fraction. Experiments with intact rats tend to support this view. The rate at which net accumulation occurs following depletion induced by ACTH seems to have the characteristics of a first-order reaction, and the velocity constant for the reaction appears to be the same or similar to the velocity constant for the steady state in hypophysectomized rats.

Methods and Materials

Intact male albino rats from the local colony, descendants of the Sprague-Dawley strain, were maintained on Purina laboratory chow and water, *ad libitum*. Range of weights was 105 to 198 grams. These animals received 6 I. U. of ACTH intraperitoneally in water per 100 grams of body weight, except for control rats, which received no further treatment. After the time periods indicated in Table and Figures below, the rats were decapitated and exsanguinated. The adrenal glands were removed, freed of adhering tissue, weighed, and homogenized in ice-cold 0.3*M* trichloroacetic acid. Ascorbic acid was measured by a scaled-up version of the micromethod of Lowry et al. (1).

Results and Discussion

Following injection of radioactive ascorbic acid, adrenal glands of hypophysectomized rats acquire radioactivity in a way which indicates the operation of a two-step mechanism (2, 3). Two maxima of specific activity occur, one much more rapidly than the other, suggesting that two fractions of ascorbic acid in adrenal glands acquire label at markedly different rates. From these data, steady-state exchange rates were obtained. It was also noted that

ACTH did not affect the rapidly-exchanging fraction to a measurable extent, the depletion of cortical ascorbic acid apparently being confined strictly to the slowly-exchanging fraction. Depletion of ascorbic acid after administration of ACTH is faster than can be expected from even the rapid step, indicating the initiation of a new, or acceleration of a constantly occurring reaction. When, however, after passage of an adequate period of time, ascorbic acid again accumulates in the adrenal gland, it does so at a rate which seems to be similar to that predictable on the basis of the steady-state measurements.

The rate K_1 at which the rapidly-exchanging fraction exchanged with extracellular ascorbic acid was found to be about 2.3×10^{-2} mg. of ascorbic acid per gram of tissue per minute (2). The rate K_2 at which the slowly-exchanging fraction acquired and lost ascorbic acid (presumably from and to the rapidly-exchanging fraction) was approximately 8.7×10^{-3} mg. per gram of tissue per minute (3). Thus it appears that K_1 may be sufficiently larger than K_2, that the concentration of rapidly-exchanging ascorbic acid should not decrease markedly even when the return from the slowly-exchanging fraction declines. This is supported by experimental evidence (2). Consequently, the concentration of rapidly-exchanging ascorbic acid may be regarded as being always approximately constant.

If the reactions leading into and from the slowly-exchanging fraction exhibit first-order kinetics, then

$$(1) \quad \frac{dA_s}{dt} = k_2 A_r - k'_2 A_s$$

and the steady state-rate $K_2 = k_2 A_r = k_2 A_{se}$, since $A_r = A_{re}$. Hence,

$$(2) \quad \frac{dA_s}{dt} = k'_2 (A_{se} - A_s)$$

and since $A_{se} - A_s = A_e - A$, A_r being constant,

$$(3) \quad k'_2 = \frac{2.303}{t} \log_{10} \frac{(A_e - A_o)}{(A_e - A_t)}$$

in which form the velocity constant may be evaluated without knowledge of the values of A_s and A_r. Plotting $\log_{10} (A_e - A_t)$ against time, a straight line should be obtained, having a slope $-k'_2/2.303$, if the reaction is of the first order[c].

Sayers et al. (5) have shown that restoration of adrenal ascorbic acid in hypophysectomized rats proceeded until (probably transiently) concentrations greater than those existing prior to

administration of ACTH were attained. This rebound effect is not predicted by equation (3). The reason for this appears to lie in the superimposition of an effect additional to simple restoration of ascorbic acid.

Adrenal glands of rats undergo atrophic changes upon hypophysectomy, involving not only a decrease in mass, but a disproportionate decrease in concentration of ascorbic acid. Reversal of this process by exogenous ACTH evidently leads to the converse situation, namely an increase in mass and a disproportionate increase in concentration of ascorbic acid. This probably constitutes the rebound effect. To avoid this complication, the use of intact rats suggests itself. In the absence of preceding atrophy, a single dose of ACTH might not lead to appreciable hypertrophy. The date of Table 1 and Figure 1, which were obtained by use of intact rats, show that this is the case. In fact, additional experiments (unpublished) failed to disclose a significant rise of concentration of adrenal ascorbic acid over the preinjection value within 45 hours of administration of ACTH.

Figure 2 represents a plot of $\log_{10}(A_\infty - A_t)$ against time, using the data from Table 1. Deviations from linearity are apparently rather grave at the longer time periods. It should be noted, however, that small errors in this range produce large deviations from linearity and that no points for values lying above A_∞ can be plotted at all since $A_\infty - A_t$ is then negative. At shorter time ranges, linearity is reasonable, once net accumulation of ascorbic acid has commenced at about 2 hours after administration of ACTH. From the slope of this curve, it is found that k'_2 for this group of rats is about .24 per hour, or 4×10^{-3} per minute. A calculated curve, describing changes in concentration of ascorbic acid with time, is shown in Figure 1. This was obtained with equation (3) and the above value for k'_2, and fits the observed relationship satisfactorily. The value for k'_2 may also be calculated for the group of hypophysectomized rats, since K_2, the steady-state rate of exchange, and A_∞, are both known (2, 3). Using these previously-reported data, k'_2 is found to be $8.7 \times 10^{-3}/2.86 = 3 \times 10^{-3}$ per minute. In view of the fundamentally different approaches used to obtain them, the close correspondence between this and the above value is gratifying. The possibility of fortuitous agreement cannot be altogether disregarded, because of the uncertainty in the underlying data, and the differ-

TABLE 1

Ascorbic Acid in Adrenal Glands of Intact Rats After Administration of ACTH

Time after ACTH (min.)	0	70	130	180	240	300	520	830	1170	1530	1800
No. of rats	14*	4	4	4	5	4	5	5	5	5	6
Adrenal asc. acid (mg./gm.)	4.50	2.40	2.44	2.72	3.12	3.45	4.18	4.29	4.48	4.29	4.56
S. D.	.34	.13	.15	.69	.45	.46	.29	.27	.35	.46	.45

* Control rats, received no ACTH

ence in animals involved in the two studies. However, if major differences existed, they would probably have become apparent.

Thus. increased permeability due to ACTH must have disappeared within two hours or, indeed, may never have occurred at all. Other evidence supports the latter idea (2). The constancy of A_r (2) and the failure of the velocity constant to be affected, both seem to indicate that loss of adrenal ascorbic acid is more apt to result from augmented rate of loss through acceleration of processes culminating in chemical transformation of ascorbic acid (4) without any change in the velocity constants describing inflow or return of slowly-exchanging ascorbic acid. It seems quite certain that it is not necessary to postulate the operation of new and mysterious processes to explain the net *accumulation* of ascorbic acid following ACTH-induced depletion. Once the probably essentially constant rate of inflow begins to exceed the transiently "abnormal" losses, net deposition could occur at a gradually diminishing rate predictable on the basis of first-order

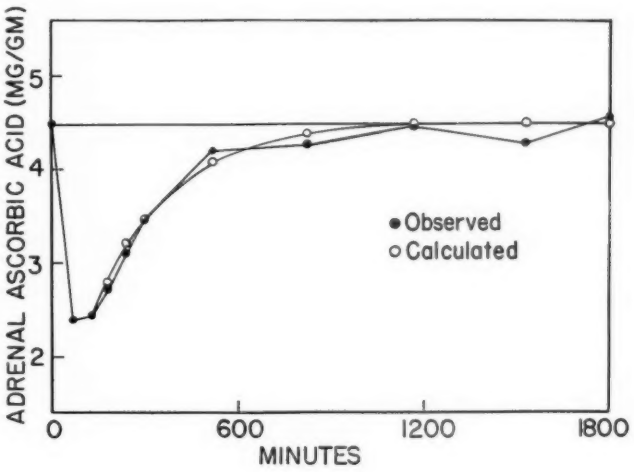

FIG. 1. Observed and calculated concentrations of adrenal ascorbic acid. Time in minutes after injection of ACTH. The concentration observed 130 minutes after injection of ACTH is taken as the starting value in the calculations.

kinetics, until return from this fraction again equals the rate of inflow. At this point, a steady-state is again attained.

Summary

Although concentrations of adrenal ascorbic acid may exceed preinjection values in hypophysectomized rats within a number of hours after administration of ACTH (5), this effect was not noted in intact rats. Using intact animals, it was shown that net accumulation of adrenal ascorbic acid follows a predictable pattern with the characteristics of a first-order reaction. The velocity constants are probably not changed by ACTH, nor do they seem to differ appreciably between intact and hypophysectomized rats. This and previous evidence fails to disclose changes in permeability due to ACTH.

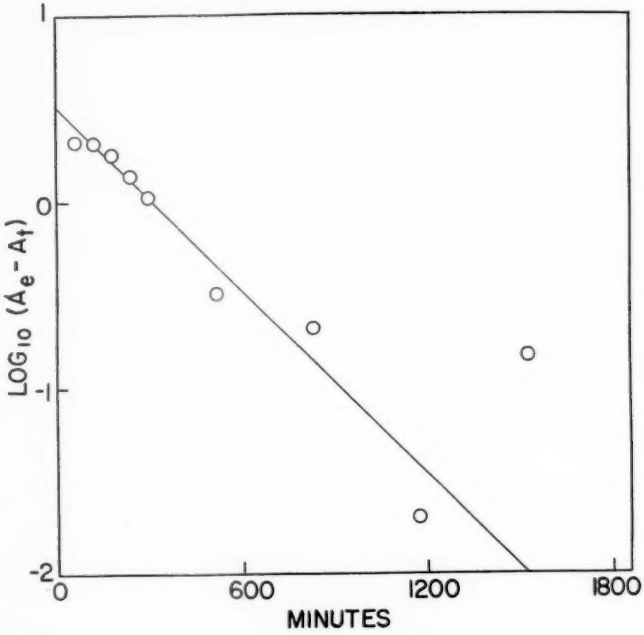

FIG. 2. Evaluation of velocity constant. Time in minutes after injection of ACTH. ($A_e - A_{1800}$) is negative, and no point is plotted for that period.

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[b] The following abbreviations were used:
A=concentration of ascorbic acid; refers to total concentration unless identified as slowly- or rapidly-exchanging.
K=steady-state rate of exchange.
k_2 and k'_2=velocity constants for flow into and form from the slowly-exchanging fraction, respectively.
t=time.
The subscripts
s=slowly-exchanging.
r=rapidly-exchanging.
e=steady-state.

[c] It is here assumed that exchange with extracellular ascorbic acid occurs *via* the rapidly-exchanging fraction, and equation (3) is based on constancy of concentration and inflow from that fraction. However, the same equation could have been derived by assuming constant inflow regardless of the source of the ascorbic acid.

WEIGHTS OF RIGHT AND LEFT KIDNEY IN DOGS[a]

H. G. SWANN AND M. J. RAILEY[b]

It is generally agreed that the two kidneys weigh almost exactly the same, but minor variation is apparent in some species. In man, the left is reported to weigh 5–7 gm.—some 4 per cent—more than the right (Mallory and Wright, 1924). The same is true for oxen (Sisson, 1921). On the other hand, in the rat (Arataki, 1926; MacKay and MacKay, 1937) and in the horse (Sisson, 1921), the right kidney weighs 3–4 per cent more than the left. Since we have been unable to find any data on this point in dogs, a study was made of the comparative weights of the two kidneys in this species. There will also be reported the relation of kidney weight to body weight in this series of animals.

Methods

Weights of the separate kidneys of 57 adult mongrel dogs were measured, the series including 27 males and 30 females. The animals were obtained from the city pound; they were in good health but otherwise unselected. The kidneys were removed while under anesthesia with sodium pentobarbital. The organs were trimmed free of fat but the capsule left intact; hilar tissues were cut away close to the body of the kidney. Before weighing, the kidneys were allowed to drain free of their contained fluids for 15 minutes.

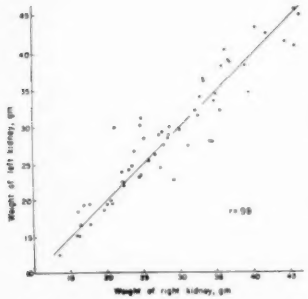

FIG. 1. Relation of right and left kidney weights.

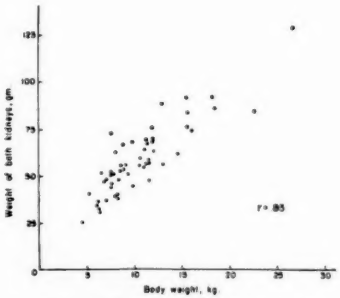

FIG. 2. Relation of combined kidney weights to body weight.

Results

The data are presented in graphic form in Figure 1. It shows that the one kidney weighs almost exactly the same as its partner for the points fall very close to the isogravimetric line. The coefficient of correlation, r, between the two is .98. The mean weight of the right kidney was 28.9 gm and that of the left was 29.4 gm, with standard deviations of 9.4 and 9.6 respectively. The difference between the two means is not statistically significant ($P > .5$).

The relation of combined kidney weights to body weight is shown in Figure 2. The coefficient of correlation between the two is .85. The line of regression for these data, assuming linearity, was calculated by the method of least squares. It yielded the relation: kidney weight is equal to .0038 times body weight plus 18.8, all weights being expressed in grams. The kidney index, i.e. the ratio of kidney weight to body weight, was calculated for the series: it averaged .00582. As more commonly phrased, the kidneys weighed 5.82 gm per kg of body weight. The extremes of this measurement were 3.76 and 9.78 gm/kg; its standard deviation was 1.18.

Discussion

It is apparent that in dogs, the two kidneys match each other in weight very closely. The left kidney is not heavier than the right as is true for man. In this series, the kidneys weighed on the average 5.82 gm per kg body weight. This is higher than the figure given by Moustgaard (1948) of about 4.7 gm, and somewhat lower than the figures of 6.7 given by Bradford (1898) or of 7.1 given by Smith (1951). The content of protein in the diet exerts a strong effect on the weight of the kidney (Smith, 1951); perhaps it is this factor that has given the present rather low kidney index, since these dogs—unclaimed dogs, obtained from a city pound—probably obtained a diet rather sparse in protein.

Summary

The left and right kidneys of 57 dogs were found to weigh almost exactly the same, the slight difference between the average weights of the two not being statistically significant. The coefficient of correlation between the two was .98. In this series of dogs the ratio of kidney weight to body weight averaged .00582.

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CATALASE ACTIVITY AND PIGMENTATION IN *SERRATIA MARCESCENS*

ROBERT P. WILLIAMS AND J. HERBERT SESSUMS

The red pigment of the microorganism, *Serratia marcescens*, has been reported to be a tripyrrylmethene structure (Wrede and Rothhaas, 1934). Studies by Williams *et al.* (1956) demonstrated that pigment identical to that of Wrede and Rothhaas could be separated into four components. However, the pigment was still assumed to have a pyrrole nature (Green *et al.*, 1956), and Castro *et al.* (1957) have reported preliminary chemical investigations which supported this assumption. In addition, Santer and Vogel (1956) demonstrated that a substance presumably containing a pyrrole ring was a precursor of the pigment.

The similarity in the pyrrole structure of the pigment to that of porphyrins prompted Crichton and Lazarus (1948) to postulate that pigment was formed by a side reaction during the biosynthesis of the porphyrin containing enzyme, catalase. The hypothesis of these investigators was based upon their observation that pigmented organisms demonstrated good catalase activity whereas nonpigmented organisms showed poor catalase activity. They proposed that pigmented strains produced an overabundance of catalase, and as the enzyme accumulated the reactions leading to its synthesis were reversed. The reversal allowed intermediate compounds of porphyrin synthesis to accumulate, and pigment was produced as a secondary reaction from one of the intermediates. In nonpigmented strains catalase was not produced, therefore the synthetic reactions were not reversed, and no intermediate compound collected to serve as a precursor to form pigment. The hypothesis involved an intimate connection between the pathways for both pigment and catalase synthesis.

The present investigation was undertaken to further assess a possible relationship between catalase activity and pigmentation in *S. marcescens*. We have employed a pigmented strain and three mutants derived from this strain. Two of these mutants exhibited the phenomenon of syntrophic pigmentation (Williams

and Green, 1956) in which one strain feeds a substance to a colorless strain causing the latter to become pigmented. The pigment formed is identical to the red pigment of the parent strain (Green and Williams, 1957). The third mutant was a stable colorless strain which would not form pigment under any conditions.

Materials and Methods

The parent organism employed in these investigations was *S. marcescens*, strain Nima. When grown at 27° C. this organism produced the red cellular pigment characteristic of the genus. The strain was extremely stable, and no colonies of a colorless nature, or of a different color than red, have appeared spontaneously when the organism was incubated at 27° C. However, when the incubation temperature was 37° C., all the colonies were colorless and white.

By treating saline suspensions of Nima with ultraviolet irradiation, it is possible to produce variously colored, as well as colorless or white, mutants. This procedure has been employed to obtain the three mutants utilized in the investigation. One of the mutants, designated OF, was orange in color and was similar in characteristics to the orange variant described by Williams and Green (1956) except that it did not pigment at 37° C. A second mutant, labeled WCF, was white in color when cultivated by itself, but became red when grown in the presence of strain OF. This phenomenon, referred to above as syntrophic pigmentation, occurred at 27° C. but not at 37° C. The third mutant, WNF, also was white in color, but it did not form pigment under any conditions tested. With the exception of pigment production, the parent and three mutant strains were identical both culturally and morphologically.

For cultivation of the organisms, a liquid medium similar to that of Williams *et al.* (1956) was utilized. The medium was dispensed in 10 ml. amounts in 50 ml. Erlenmeyer flasks. Inocula consisted of 0.1 ml. of 24 hr. cultures grown in the same medium. The organisms were grown without shaking for 48 hrs. at 27° C. or 37° C., depending upon whether pigmented or nonpigmented cells were to be tested. After incubation, the cells were harvested by centrifugation, and washed three times with 0.1 molar phosphate buffer, pH 7.0.

Catalase activity was measured manometrically in a Warburg apparatus employing hydrogen peroxide as the substrate. Preliminary investigations were carried out to determine the proper cell concentration, pH, temperature, and hydrogen peroxide concentration to employ for Warburg experiments. A pH of 7.0, temperature of 30° C., and a hydrogen peroxide concentration of 1.94 millimols were selected as giving the best response. The hydrogen peroxide was diluted in 0.1 molar phosphate buffer, pH 7.0, and added in 2.2 ml. of solution to the main compartment of the Warburg vessel.

The washed, packed cells from 10 ml. of media were diluted 1:100 in 0.1 molar phosphate buffer, and 1.0 ml. of the suspension added to the side arm of the Warburg vessel. To express the results in a uniform manner, micro-

Kjeldahl analyses were carried out on each cell suspension. The amount of nitrogen contained in 1.0 ml. of the various cell suspensions was approximately 0.1 mg. All results are reported as the microliters of oxygen evolved per 0.1 mg. of bacterial nitrogen.

Several experiments were performed on each organism. Control flasks containing only hydrogen peroxide or cell suspensions were included in each experiment. The controls demonstrated only slight manometric changes, and these changes were incorporated in the experimental calculations. The data are reported as corrected figures.

Results

The catalase activity of the various strains was determined over a period of 1 hr. The greatest oxygen evolution occurred during the first 30 min. of incubation. In Figure 1 are presented typical results of catalase determinations carried out on the four strains grown at 27°C. At this temperature both Nima and OF strains produced abundant pigment, and both organisms demonstrated catalase activity. Strain WCF, which is white when grown by itself, but will form red pigment when grown together with OF at 27°C., also possessed catalase activity.

The poorest response of organisms grown at 27°C. was always shown by strain WNF. This organism will not form pigment under any conditions. However, although the response was poor, the organism always demonstrated some catalase activity.

Typical results of experiments carried out employing organisms grown at 37°C. are presented in Figure 2. At this incubation temperature none of the strains formed pigment. However, it is evident that both Nima and OF strains, which produced pigment when grown at 27°C., showed no diminution of catalase activity when grown as nonpigmented organisms at 37°C. As a matter of fact, Nima possessed a 30–40 per cent greater activity when grown at the higher temperature. Strain WCF also showed no decrease in catalase activity when grown at 37°C.

Of particular interest is the response of the nonpigmented strain, WNF. When grown at 37°C. this organism demonstrated no catalase activity. To verify this observation, heavy suspensions of washed WNF cells grown at 37°C. were spread on the surface of evaporating dishes. A 33 per cent solution of hydrogen peroxide was added drop by drop to the cells, and the reaction observed. No evidence of gas formation was seen under these conditions. When the same experiment was repeated employing

either Nima, OF, WCF, or WNF cells grown at 27°C., there was a marked evolution of gas as evidenced by frothing and foaming of the added hydrogen peroxide solution. Thus it was evident that when strain WNF was grown at 37°C. the cells possessed little or no catalase activity.

Discussion

Our results demonstrate that there is no definite relationship between catalase activity and pigmentation in *S. marcescens*.

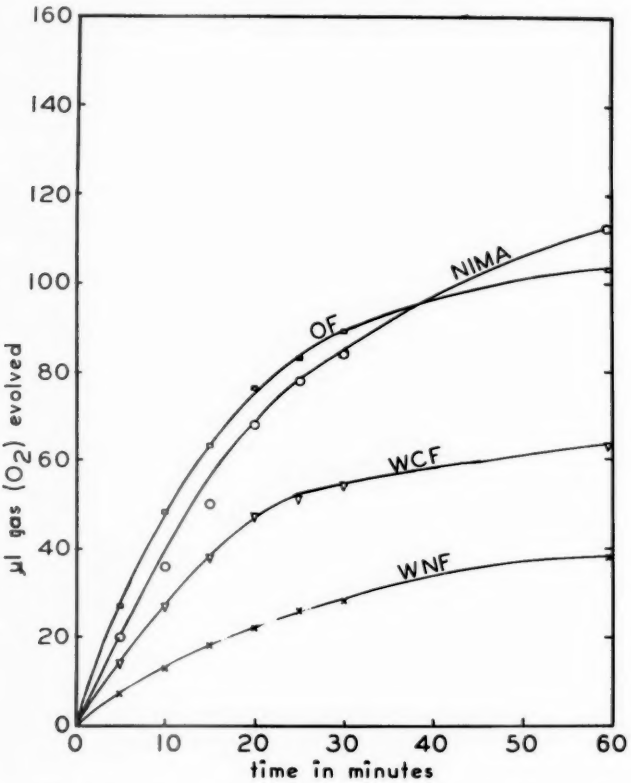


FIG. 1. Catalase activity of *Serratia mercescens* strains when grown at 27° C. for 48 hrs. Activity is expressed as microliters of 0_2 evolved per 0.1 mg. of bacterial nitrogen.

strain Nima. Thus the hypothesis of previous investigators (Crichton and Lazarus, 1948) which correlated pigment synthesis with visible pigmentation and catalase activity is not tenable. Organisms which form pigment when grown at 27°C. do have catalase activity, but the activity is still present when the organisms are grown at 37°C. where no pigment is formed.

However, the results obtained with the two white mutants, WCF and WNF, suggest that there might be a more subtle re-

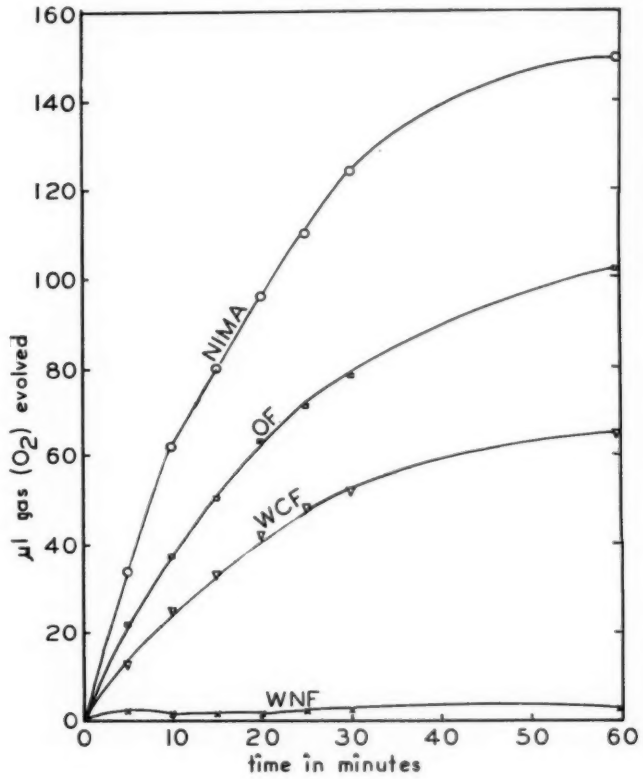


FIG. 2. Catalase activity of *Serratia marcescens* strains when grown at 37° C. for 48 hrs. Activity is expressed in the same manner as in Figure 1.

lationship between pigment synthesis and catalase activity. Strain WCF does not form pigment when grown by itself, but will pigment when grown in the presence of strain OF. Thus WCF retains the ability to form pigment provided the proper precursor is present. This organism possesses catalase activity whether grown at 27°C. or 37°C., although its ability to synthesize pigment is lost at the latter temperature. Strain WNF has completely lost the ability to form pigment. When grown at 27°C. this organism possesses some catalase activity, but when grown at 37°C. the activity is absent.

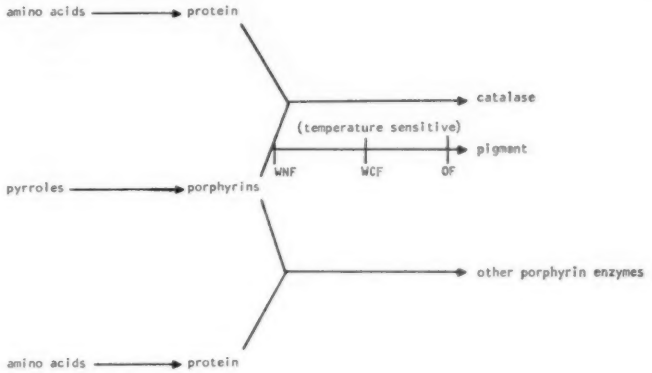
From the above facts one can speculate that catalase activity and the ability to form pigment might be related, and that the reactions are temperature sensitive at a point early in the biosynthetic pathway. Such a speculation would envision mutant WCF as blocked at a point where pigment synthesis was affected, but not catalase synthesis. Mutant WNF would be blocked at a point early in the pathway of pigment biosynthesis, and the mutation also would have a temperature effect upon catalase synthesis. The latter reaction would be completely blocked when the organisms were grown at 37°C. Temperature sensitive reactions have been described for mutants of other organisms (Wagner and Mitchell, 1955).

We believe the hypothesis of Crichton and Lazarus (1948) is weak because in addition to a lack of experimental proof it fails to consider other porphyrin requiring enzyme systems of the organism. According to their scheme, an organism which was not pigmented would have lost the ability to form the porphyrin moiety of catalase. Since the porphyrins also constitute the prosthetic groups of other enzyme systems, such as the cytochromes, one would also expect the function of these enzymes, to be affected by a mutation which would hinder porphyrin synthesis. Cytochrome function does not appear to be affected in the organisms employed in the present investigation. Both pigmented and colorless strains grow best aerobically, growth is augmented by shaking, and the cell suspensions demonstrate marked oxygen uptake when glucose is the substrate. Therefore the organisms appear to possess the cytochrome enzymes required for aerobic respiration.

Our proposed scheme of pigment synthesis, and its relationship to catalase activity, would place the mutation completely

inhibiting pigment synthesis (strain WNF) after the formation of porphyrin, but before the porphyrin is linked to a protein apoenzyme to form catalase. The mutation present in strain WCF would occur along the pathway for pigment biosynthesis, and would not affect the catalase system. Pigment synthesis would be carried out by temperature sensitive reactions. The reaction blocked in WNF would be located near the point of divergence between pigment and catalase synthesis, and the temperature sensitivity in the mutant of the latter reaction would be an indication of the close relationship of the two pathways.

The proposed scheme is outlined in the following diagram. The points at which the various mutants are blocked in their synthesis of pigment are indicated. Since mutant OF accumulates a substance which serves as a precursor for pigment formation in WCF, the mutation occurring in OF is placed beyond the point of mutation in WCF.



Summary

The catalase activity of *Serratia marcescens*, strain Nima, and three of its mutants was measured manometrically. There was no correlation between catalase activity and visible pigmentation or incubation temperature of the organisms. However, the results obtained with two white mutants suggested that there might be a positive correlation between catalase activity and the ability to form pigment. On the basis of these observations a new scheme of pigment biosynthesis was proposed.

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ERYTHREMIC MYELOSIS

Summary of the Literature and Report of a Case

Klaus Bensch, M.D., R. H. Rigdon, M.D.*,
and Charles Phillips, M.D.

To the Italians must go the recognition for the disease we now designate as either erythroblastemia, erythremic myelosis or DiGuglielmo's disease. The first case was reported in 1912 by Copelli. DiGuglielmo in 1926 first recognized the disease as an entity. In 1938 Baserga collected from the literature 29 cases that he considered to be erythremic myelosis. Moeschlin in 1940 accepted five as erythremic myelosis; Schwartz and Critchlow in 1952 accepted ten (Benedetti, 1939; Copelli, 1911–12; DiGuglielmo, 1928; DiGuglielmo and Quattrin, 1942; Duesberg, 1940; Heilmeyer and Schoner, 1941; Israels, 1939; Lazzaro, 1933; Pittaluga, 1940; Stodtmeister, 1941) and added four of their own. In our opinion the case reported by Fitzgerald et al. in 1950 should be added to this group of fourteen cases selected by Schwartz and Critchlow (1952), giving a grand total of fifteen cases reported as of this date.

Many cases considered to be erythremic myelosis or related to it have been reported since the review by Baserga (1938). Excellent discussions of these problems may be found in the papers by Vaughan (1936), Moeschlin (1940), Heilmeyer and Schoner (1941), and Fitzgerald et al. (1950). Schwartz and Critchlow in 1952 gave an excellent review of this disease. They pointed out that the term "erythremic myelosis" is used to indicate the parallelism with the leukemic myelosis. Erythremic myelosis apparently represents a neoplastic process of the cells in the erythrocytic series. DiGuglielmo (1946) regarded the disease as one of hyperplasia, anaplasia, and dysplasia of the erythropoietic system affecting the bone marrow, liver, spleen, and the extra hematopoietic tissues. Primitive red cells were produced that were atypical and fragile. Their maturation was abnormal, resulting in the anemia. Dameshek (1951) suggested that the cell response in erythremic myelosis may be elicited along multiple channels rather than in only one as suggested

by DiGuglielmo. Schwartz and Critchlow (1952) thought the theory suggested by Dameshek was the simplest to comprehend if one accepts the "stem" cell to be multipotential.

Erythremic myelosis is characterized by either an acute or chronic course (Schwartz and Critchlow, 1952), the former varying in duration from a few weeks to two months and the latter lasting about two years. A severe anemia, irregular fever, splenomegaly, and usually hepatomegaly are the typical symptoms of the disease. In the acute form the red cell count is usually between 1.0 and 2.5M. Nucleated red cells are numerous in the peripheral blood. Atypical forms are usually conspicuous. Basophilic erythroblasts predominate over the more mature forms in the peripheral blood in the acute form of the disease, while the reverse is usually true in the chronic (Schwartz and Critchlow, 1952). The white cell count may be as high as 30,000 per cu. mm. A summary of the clinical and pathologic findings in the fourteen cases of erythremic myelosis accepted as typical by Schwartz and Critchlow (1952), the case reported by Fitzgerald et al. (1950), and ours are shown in Table 1.

Case Report

The patient was a 38-year-old colored female hospitalized for abdominal swelling, swelling of ankles, weakness, and a low grade fever of one month's duration. During this time there was menstrual irregularity characterized by irregular bleeding and the passing of clots. Since the age of eighteen the patient had hirsutism which necessitated regular shaving. There had been four pregnancies and one miscarriage. Leg ulcers were present for the past six years.

On physical examination this woman was lethargic and appeared acutely ill. The temperature was 100° F., P. 108, R. 22, and the blood pressure was 110 mm. of Hg. systolic and the diastolic pressure was 70 mm. of Hg. The spleen was markedly enlarged, the liver was questionably enlarged, and a few small lymph nodes were present in the axillary and cervical regions. There was a beard, coarse axillary hairs, large hands, and a prominent jaw. The voice was normal.

Laboratory data: All the blood and platelet counts and the amount of hemoglobin present during the eight weeks of hospitalization are given in Table 2. Howell-Jolly bodies were present.

TABLE 1

Summary of the Findings in 16 Cases of Erythremic Myelosis

Case	Autopsy	Year	Age	Sex	Race	Duration	Nucleated RBC per 100 WBC	WBC	RBC	Hemoglobin	Platelets	Spleen	Liver	Bone Marrow
DiGuglielmo	+	1928	50	M	W	7 wks.	...	4,000	1.3	40%	Reduced	1,700	Large	Immature Erythroblast
Lazzaro	+	1933	23	M	W	Short	...	....	2.1	29%	8,000	550	2,310	Hyperplastic Erythroblast
Stodmeister	+	1941	37	F	W	Short	1.4	7,200	0.9	25%	Reduced	1,050	Large	Hyperplastic Erythroblast
Copelli	+	1912	60	M	W	2 yrs.	...	2,500	0.8	16%	.....	Large	....	.....
Benedetti	+	1938	46	F	W	6 yrs.	...	1,800	1.7	35%	110,000	Large	Large	Hyperplastic Erythroblast
Israels	0	1939	42	F	W	1 yr.	...	34,000	3.3	45%	149,000	Large	Large	Hyperplastic Erythroblast
Pittaluga	+	1940	42	M	W	9 mos.	20	4,000	2.2	65%	100,000	Large	Large	Hyperplastic Erythroblast
Duesberg	+	1940	50	M	W	2 yrs.	5	1,000	...	30%	50,000	850	2,020	Hyperplastic Erythroblast
Heilmeyer and Schoner	+	1941	75	M	W	2.5 yrs.	40	5,200	2.1	45%	.....	Large	Large	Hyperplastic Erythroblast
DiGuglielmo and Quattrin	+	1942	12	F	W	2 yrs.	125	12,590	0.9	20%	900,000	Large	Large	Hyperplastic Erythroblast
Fitzgerald, Mallory, and Parker	+	1950	45	F	W	1 mo.	112	5,900 to 38,000	2.2	7.5 Gm.	Reduced	750	2,260	.....
Schwartz and Critchlow	+	1944	47	F	N	8 yrs.	...	11,000 to 48,000	0.7	12%	200,000	....	3,160	.....
Schwartz and Critchlow	+	1944	49	F	W	3 yrs.	...	10,400	1.4	22%	125,000	520	....	Hyperplastic Erythroblast
Schwartz and Critchlow	+	1947	56	F	W	6 mos.	...	2,500	2.2	41%	.....	250	1,800	Hyperplastic Erythroblast
Schwartz and Critchlow	+	1948	34	M	W	1 yr.	...	9,200	1.1	21%	63,420	Large	Large	Hyperplastic Erythroblast
Bensch, Rigdon, and Phillips	+	1956	38	F	N	10 wks.	11 to 225	11,000 to 48,000	2.5	6 Gm.	50,000	780	2,490	Hyperplastic Erythroblast

TABLE 2

Hematologic Findings and Time Transfusions Were Given During Hospitalization

Date 1955	Platelets	Total Nucleated Cells	RBC	Hb	Nucleated RBC per 100 WBC	WBC	Normal														
							Polys	Stabs	Lymph	Mono	Baso	Meta	Myelocyte	Promyelocyte	Blast	Promono	Immat. Lymph	Atypical Lymph	Reticulocytes	Transfusions cc. of Blood
4/14	64,000	11,250	2.8	6.7	11	10,100	50	8	37	5	..	..	..	..	..	..	..	..	...	....
4/20	66,000	20,000	2.9	7.5	47	13,600	41	18	19	16	..	2	4	..	..	..	..	..	8.9%	....
4/22	46,000	16,000	2.0	6.0	73	9,200	36	20	13	8	1	6	5	2	1	8	..	..	...	....
4/25	40,000	19,000	1.6	5.7	115	8,800	33	19	22	3	..	5	..	7	10	1	..	..	...	500
4/27	24,000	25,000	2.0	7.0	118	11,400	8	20	..	10	..	8	4	4	10	..	12	24	...	500
4/28	32,000	20,000	2.5	7.8	121	9,000	33	18	22	2	1	8	4	3	6	..	2	1	15.1%	500
4/29	30,000	18,000	2.4	7.8	68	10,800	39	21	10	8	1	3	5	2	4	1	6	..	...	500
5/2	56,000	36,000	4.9	5.9	80	20,000	21	12	32	1	..	1	1	..	1	1	7	23	...	....
5/4	20,000	43,000	2.0	6.0	125	19,100	15	28	28	1	..	..	7	1	..	2	10	8	...	....
5/6	20,000	46,000	1.8	5.8	140	19,100	30	7	38	3	..	..	3	1	1	4	4	9	...	1,000
5/9	22,000	63,000	2.0	6.4	71	36,800	20	18	52	3	1	1	2	2	..	..	..	1	...	1,000
5/11	22,000	23,000	2.0	6.7	123	10,300	27	23	27	8	..	2	2	..	..	1	..	10	...	1,000
5/13	.....	48,000	2.6	7.7	123	21,500	38	22	24	5	1	5	1	..	..	..	..	4	...	500
5/16	56,000	44,500	3.4	9.8	71	26,000	44	11	16	10	..	4	2	2	1	1	1	8	...	500
5/20	34,000	31,000	2.0	6.5	76	17,700	38	30	14	8	..	1	5	2	..	..	..	2	...	1,000
5/23	62,000	14,000	4.8	4.8	88	7,500	64	14	10	6	..	1	3	2	..	.	..	..	...	1,000
5/25	22,000	22,000	1.5	5.2	171	8,100	61	22	10	5	..	1	..	1	..	..	..	..	...	1,000
5/27	40,000	22,400	0.9	3.9	225	6,800	48	33	11	5	..	2	1	..	..	..	..	..	...	1,000

Many spherocytes were present in the peripheral blood. Fragility tests showed an initial hemolysis at 0.42 per cent NaCl and complete hemolysis at 0.28 per cent. A sickle cell preparation was negative. The total protein was 6.4 gm. per 100 ml. with 3.8 gm. of albumin and 2.6 gm. of globulin. The alkaline phosphate was 2.3, thymol turbidity 6.3, and the serum bilirubin was 1.2 mg. per cent. The iso-agglutinins on the 12th day showed an anti B titre of 1 to 64, the iso-hemolysin titre was 1 to 2 and the cold agglutinin titre was 1 to 8. The urine urobilinogen on the 1st day of hospitalization was positive in a dilution of 1/40 and a trace was present at 1/80. The bones were normal roentgenologically.

A surgical marrow biopsy from the sternum was taken on the 6th day of hospitalization. It showed a hyperplastic marrow composed of both erythroid and reticulum cells. A lymph node removed from the left axilla on the 12th day showed only hyperplasia of the reticulum cells. The platelet counts during hospitalization (Table 2) varied between 20,000 and 66,000 with an average of 36,000. The white blood cell counts varied between 11,250 and 48,000 with an average of 30,000. The red blood cell counts varied between 1.54 and 4.9 M. and the hemoglobin between 5.2 and 7.8 G. Nucleated red cells were prominent in every blood count, varying from 11 per 100 white blood cells on admission to 225 per 100 white blood cells on the 3rd day before her death. Obviously the total number of nucleated cells in the peripheral blood was significantly increased with such a high percentage of erythroblasts and nucleated red cells. The most significant feature in the differential leucocyte counts was the presence of "blast" cells and the so-called immature and atypical lymphocytes.

The patient received 10 liters of blood (Table 2), and cortisone while in the hospital. On the 21st hospital day she had a spontaneous hemorrhage from Kieselbach's area which was controlled by cauterization. She failed to respond to therapy and died on the 40th day after admission.

The postmortem was performed four and one-half hours after death. The final anatomic diagnoses are:

1. Erythremic myelosis (clinical history of refractory anemia with 11 to 225 nucleated red bood cells per 100 white blood cells in peripheral blood).

2. Erythroid and myeloid hyperplasia of the marrow from multiple bones.
3. Proliferation of young reticulo-endothelial-like cells in the spleen, liver, and lymph nodes.
4. Focal areas of extra medullary hematopoiesis in liver and spleen.
5. Splenomegaly (780 gms.) with old and recent infarcts.
6. Hepatomegaly (2490 gms.) with focal areas of necrosis.
7. Ascites, 4000 ml.
8. Pulmonary edema.
9. Two gastric ulcers, 1.2 and 1.4 cms. in diameter.

In reviewing the smears from the peripheral blood many "blasts" are present and they are difficult to classify. The chromatin in these cells is irregular and stains dark blue. There is no nucleolus and very little cytoplasm; when the latter is present it stains blue. There is a wide variation in the size of the normoblasts. The red cells are polychromatic, hyperchromatic, and show Howell-Jolly bodies. Spherocytes are present. Smears from the bone marrow made at the autopsy show many "blasts" (Figures 1 and 2). The marrow in the histologic section is hyperplastic. The majority of the cells in the marrow are large mononuclear cells with coarse strands of chromatin and little, if any, cytoplasm (Figure 3). Very few megakaryocytes are present. Large mononuclear cells, like those in the hyperplastic marrow, are present in the splenic sinuses (Figure 4) and in the sinusoids of the liver (Figure 5). There are focal areas in the liver (Figure 6) in which these cells completely replace the hepatic tissue, while in other areas the hepatic sinusoids are filled with these cells (Figure 7). The lymph nodes are hyperplastic; however, the number of these "blast" cells in the lymph nodes are few when compared with those in the bone marrow, liver, and spleen. Large foci of hematopoiesis are present in the liver and spleen (Figure 8). Many small areas of necrosis and fibrosis are present in the spleen consistent with recent and old infarcts.

The only pathologic findings of interest in this case, other than those related to the primary disease, are two ulcers in the pyloric end of the stomach along the lesser curvature. The base of each ulcer is granular, suggesting chronicity. The contents of the colon are black in color, suggesting old blood. No recent hemorrhage is present in the gastro-intestinal tract; in fact, there are no hemorrhages in any of the viscera.

EXPLANATION OF ILLUSTRATIONS

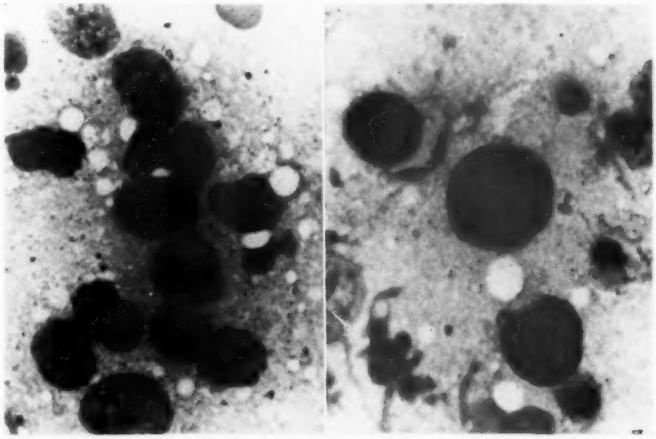

FIG. 1. The bone marrow is hyperplastic. Many erythroblasts are present. This illustrates the type of cell frequently found in a smear of the marrow from a rib obtained at time of autopsy. Wright's stain, X 1615.

FIG. 2. Erythroblasts and nucleated red blood cells in smear of marrow from vertebrae. Wright's stain, X 1615.

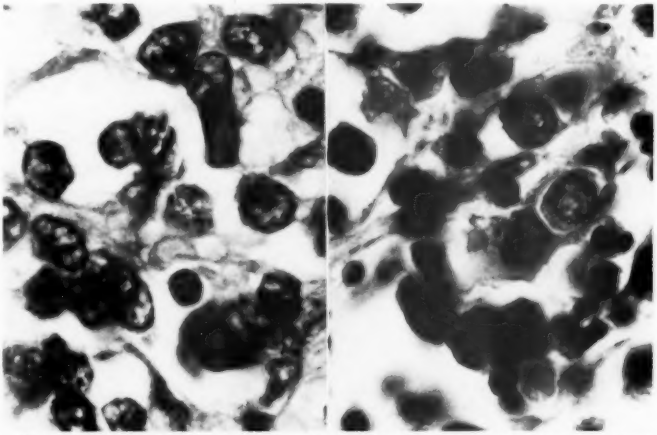

FIG. 3. In the histologic section of bone marrow from the sternum there are many large "stem"-like cells in which there are interlacing strands of chromatin. The cytoplasm is either minimal or absent. These cells are considered most likely to belong to the erythroid series. H&E, X 1615.

FIG. 4. There are many cells in the sinuses of the spleen that are similar to those in the bone marrow. H&E stain, X 1615.

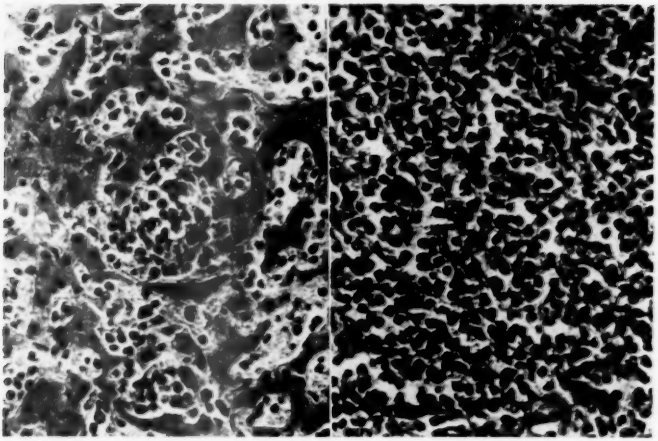

FIG. 5. The hepatic sinusoids are filled with blast-like cells similar to those present in marrow and spleen. H&E stain, X 340.

FIG. 6. In focal areas of the liver the blast-like cells have proliferated to such an extent that the hepatic cells are completely absent. H&E stain, X 340.

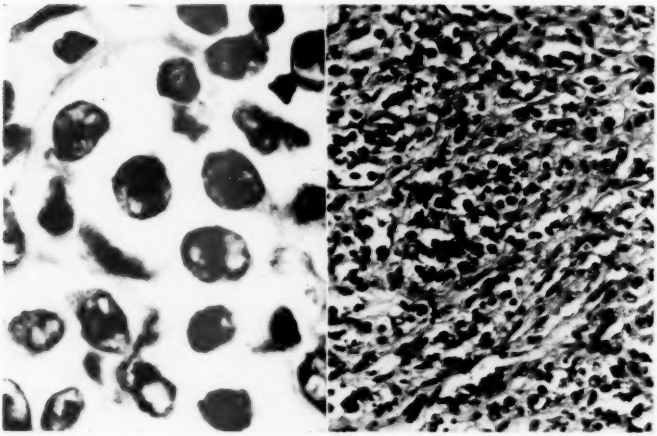

FIG. 7. The blast-like cells in the hyperplastic nodules in the liver are similar to those in marrow and spleen as shown in Figures 3 and 4.

FIG. 8. Areas of extramedullary hematopoietic tissue are present in liver and spleen. This illustrates one of the areas in the spleen. H&E stain, X 340.

Discussion

The clinical, hematologic, and pathologic changes in the case reported are similar to those found in the acute form of erythremic myelosis and described as typical in a review of this disease by Schwartz and Critchlow (1952). Our case is very similar to the one reported in 1950 by Fitzgerald et al. It is of interest to note the severe anemia in our case and the failure of the patient to respond to multiple transfusions. Only once during hospitalization did bleeding occur; this was from Kieselbach's area and was easily controlled by cautery. No petechiae were present at autopsy. Patients with erythremic myelosis apparently do not show a bleeding tendency similar to that occurring in typical cases of leukemia although their platelets are usually decreased. The platelet counts in our case varied between 20,000 and 66,000.

The number of nucleated red cells in the peripheral blood varies during the course of the disease in each individual case, as well as in different cases of erythremic myelosis. In our case there were 11 nucleated red blood cells per 100 white blood cells when the patient was first seen. This number progressively increased to 225 per 100 white blood cells on the third day preceding the time of her death. Other than for a few basophils, metamyelocytes, myelocytes and promyelocytes in the peripheral blood, the most conspicuous cells were classified as blasts, promonocytes, immature and atypical lymphocytes. Diggs et al. (1956) has emphasized that "in many individual cells the similarity between plasmocytes, prorubricytes and lymphocytes is so close that differentiation cannot be made on morphological grounds alone." In view of this well recognized fact, it is our opinion that these atypical cells in the peripheral blood of our case belong to the erythroblastic rather than to the lymphoid series. There is nothing in our case to suggest lymphoid leukemia. Furthermore, the total number of white blood cells and the differential count would not support the diagnosis of myeloid leukemia. In the case reported by Israels (1939) there were 43.5% polymorphonuclear leukocytes, lymphocytes 12.5%, mononuclear 4%, eosinophils 1%, metamyelocytes 9%, myelocytes 28%, and myeloblasts 1.5% and in the case reported by Pittaluga (1940) there were 69% polymorphonuclear leucocytes, eosinophils 2%, mononuclear 9%, lymphocytes 14% and promyelocytes 6%.

The number of white blood cells in the peripheral blood in erythremic myelosis is usually low; however, it may be either normal or high (1952). The number of white cells as reported in our case varied between 11,250 and 63,000; however, this number would be significantly decreased if the nucleated red cells were withdrawn from the total counts as shown in Table 1. The actual white blood cell count would then vary between 6,800 and 36,800 with an average of 14,750.

The pathologic findings in cases of erythremic myelosis resemble those of leukemia, and without the clinical and hematological data to support such a diagnosis it would be difficult and probably impossible to make a diagnosis from only the postmortem findings. In addition to the typically enlarged spleen and liver and the hyperplasia of the bone marrow, there is an infiltration of both primitive red cells and reticulo-endothelial cells in the kidneys, adrenals, myocardium, lungs, pancreas, testes, uterus, larynx, trachea, and dura mater (1952). A case considered to be erythremic myelosis and reported by Mariotti in 1935 had multiple gastric ulcers. Our case also had two gastric ulcers. These are the only two cases we have found of this disease accompanied by this gastric lesion.

Neoplasia, as illustrated by leukemia, frequently occurs in the myeloid series of cells, less frequently in the so-called "stem" cell, infrequently in the megakaryocytes, and very rarely in the erythroblastic series of cells. Only fifteen cases have now been reported of neoplasia originating from the erythroid elements. Ten of these are of the chronic form and four are of the acute type as classified by Schwartz and Critchlow (1952). The case reported by Fitzgerald and associates (1950) and ours may be considered as acute, giving a total of six acute and ten chronic cases.

Summary

The literature is given on erythremic myelosis and associated diseases. Sixteen cases of erythremic myelosis have been reported since 1912. Six of these may be considered as acute and ten as chronic. Death occurred in the former group within an interval of three months, while those with the chronic form survived for two to three years. A case of erythremic myelosis in a 38-year-old

colored female is reported. Death occurred approximately ten weeks following the onset of weakness associated with a low grade temperature. Three days before death the red blood cell count was 0.91 M., hemoglobin 3.0 gm., white blood cells 22,400, platelets 40,000, and 225 nucleated red blood cells per 100 white blood cells were present.

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RADIOLOGICAL CASE OF THE WEEK

MELVIN H. SCHREIBER

The patient is a 13-year-old white female with the complaint of "back trouble" since the age of 6 years. She currently complains of pain in the back for the past 3 or 4 months. In addition she has frequency of urination, nocturia, and occasional burning dysuria. Physical examination shows kyphosis and tenderness to palpation in both flanks. A chest X-ray reveals bilateral hilar calcifications. and the urine analysis shows gross pyuria.

(The problem film is part of an intravenous urogram. The black arrows point to the kidney collecting systems.)

The problem film, part of an intravenous urogram, shows both kidney collecting systems to be opacified. Although incompletely filled, no gross abnormality is seen.

The film also demonstrates extensive destruction of the first three lumbar vertebrae, with partial collapse and with reactive sclerosis. Her kyphosis may be attributed to this vertebral deformity, which represents the end stage of tuberculous spondylitis.

Beneath the collecting system of each kidney is seen a partially calcified roughly oblong structure. These represent calcified psoas abscesses.

Almost half of the cases of skeletal tuberculosis involve the spine, and the lower thoracic and upper lumbar region is said to be most frequently involved. As the granulomatous destructive process progresses some degree of vertebral collapse is almost inevitable, with a gibbus deformity appearing at the affected level.

Cold abscesses form in the majority of patients with vertebral tuberculosis. As they increase in size they frequently dissect through tissue planes to some location where they may penetrate the skin and create a sinus. At the lumbar level, these abscesses frequently follow the psoas sheath and may point beneath the inguinal ligament in the groin or adjacent to the anterior superior iliac spine. The rather slowly progressive and indolent course of this granulomatous inflammatory process allows time for dystrophic calcification to occur, as in the case in point.

Acid fast bacilli were seen on smears of the patient's urine, and Mycobacterium tuberculosis was isolated in culture and from a guinea pig innoculated with the patient's urine. The final diagnoses included renal tuberculosis, active, tuberculosis spondylitis, and bilateral calcified tuberculous psoas abscesses.

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Fundamentals of Clinical Orthopedics, Peter A. Casagrande and Harold M. Frost, Jr., Grune and Stratton, New York, 1953, page 167.

CALLING ATTENTION TO

December, 1958

Holiday Books: DT Atkinson's *Magic, Myth & Medicine* is reprinted paperback (Premier, NY 36, 1958, 240pp, 50c). LG Blochman well describes *Doctor Squibb: The Life & Times of a Rugged Idealist* (Simon & Schuster, NY, 58, 358pp, $5). DJ Boorstin takes new look at influence of *The Americans: The Colonial Experience* (Random House, NY, 58, 460pp, $6). R Carter probes *The Doctor Business*, analysing AMA influence (Doubleday, NY, 58, 283pp, $4). L Cottrell tells about *The Bull of Minos* & Cretan archeology (Rinehart, NY, 58, 314pp, $4.5). C DeWitt examines *Privileged Communications between Physician & Patient* (CC Thomas, Springfield, Ill, 58, 548pp, $12). WC Gibson respects *Young Endeavor: Contributions to Science by Medical Students* (CC Thomas, Springfield, Ill, 58, 292pp, $6). A Huxley is wise in *Brave New World Revisited* (Harper, NY, 58, 147pp, $3). R Jungk notes what is *Brighter than a Thousand Suns: Personal History of Atomic Scientists* (Harcourt, NY, 58, 278pp, $5). TE Keys enjoys *Applied Medical Library Practice* (CC Thomas, Springfield, Ill, 58, 522pp, $11). CA Larson has fun in *Who: 60 Years of American Eminence* (McDowell-Obolensky, NY, 58, 387pp, $5). D. Lavender adds to Mainstream of America series *Land of Giants* (Doubleday, Garden City, NY, 58, 410pp, $6). CD Leake's 1928 translation of Harvey's *De Motu Cordis* goes into 4th edition (CC Thomas, Springfield, Ill, 58, 156pp, $2 since no royalties). S&V Leff look *From Witchcraft to World Health* (Macmillan, NY, 58, 236pp, $4.5). MS Marshall praises *Crusader Undaunted: Dr. JC Geiger. Private Physician to the Public* (Macmillan, NY, 58, 246pp, $3.5). G Murphy enthuses over *Human Potentialities* (Basic Books, NY, 58, 340pp, $6). OL Reiser tries *The Integration of Human Knowledge* (Porter Sargent, Boston, 58, 478pp, $8). M Renault well tells of Theseus in *The King Must Die* (Pantheon, NY, 58, 389pp, $5). Soviet silliness well helps sell B Pasternak's epic *Doctor Zhivago* (Pantheon, NY, 58, 876pp, $5). B. Pasternak's *Safe Conduct* is also available (New Directions Paperbook, NY, 58, 234pp, $1.35). AB Saarinen describes off-beat art collectors in *The Proud Possessor's* (Random House, NY, 278pp, $6). W Seagle raises questions in *Acquitted of Murder* (Regnery, Chicago, 345pp, $5). You'll enjoy *Alec Templeton's Music Boxes* (W Funk, NY 10, 58, 220pp, $4). S Zimand edits papers of Homer Folks on *Public Health & Welfare: Citizens Responsibility* (Macmillan, NY, 58, 475pp, $7).

Drug Advertising: Pfizer Laboratories may help restore sanity & decency to drug advertising in medical journals by example set in *Report on Diabinese*, an informative review in front ad section of Nov 22 *JAMA*, referring to oral hypoglycemic chlorpropamide, chlorobenzene-sulfonyl-propylurea (see SB Beaser, *New Eng J Med* 259/573/Sept 18/58; I Murray & Co, *Lancet* 2/553/Sept 13/58 & KM West & SR McCampbell, *Proc Soc Exp Biol Med* 98/724/58). OK, Pfizer, now keeps the ads soft-sell & informative.

Clinical: EV Cowdry edits *The Care of the Geriatric Patient* (CV Moseby,

St. Louis, Mo 58, 438pp, $8). HF Harlow edits *Biological & Biochemical Bases of Behavior* (Univ Wisconsin Press, Madison, 58, 348pp, $8). EG Jaco edits *Patients, Physicians & Illness: Behavorial Science & Medicine* (Free Press, Glencoe, Ill, 58, 600pp, $7.5). S. Liebman edits *Emotional Problems of Children* (JB Lippincott, Philadelphia, 58, 176pp, $5). G Owens suggests fat embolism as factor in ether & heat convulsions (*Neurol* 8/827/58). CM Plum indicates importance of electrolyte variations in cerebrospinal fluid in pathological conditions (*Acta Psychiat Neurol Scand* 33/477/58). L Szondi & Co describe *The Szondi Test in Diagnosis, Prognosis & Treatment* (JB Lippincott, Philadelphia, 58, 346pp, $12). HE Ungerleider & RS Gubner analyse *Life Insurance & Medicine* (CC Thomas, Springfield, Ill, 58, 1108pp, $16.5). J Van Huf-Van Duin analyses adynamia in adrenocortical insufficiency (*Acta Physiol Pharmacol Neerland* 7/289). J Youmans edits symposium on nutrition in internal medicine (*Amer J Med* 25/659–732/58). CB Young describes wise *Transportation of the Injured* (CC Thomas, Springfield, Ill, 58, 256pp, $7).

Physiology & Pharmacology: WO Cruz & A Baumgarten suggest canine blood incompatibilities (*Acta Physiol Lat-Amer* 8/11/58). R Edstrom suggests blood-brain barrier phenomena influenced by lack of extravascular space (*Acta Psychiat Neurol Scand* 33/405/58). T Erdos & Co study benzoic acid & catechol oxidases (*Acta Physiol Acad Sci Hungar* 14/203, 213/58). PAJ Janssen & Co find potent norpethidine analgesics (*Acta Physiol Pharmacol Neerland* 7/373/58). AV Muralt gives *Neue Ergebnisse der Nervenphysiologie.* MF Perutz notes advances in molecular biology (*Endeavor* 17/190/58). A Pirie analyses biochemistry of eyes (*ibid* p 181). LJ Rather notes significance of nuclear size in pathological processes (*Ergebnisse allg Path* 38/161/58). D Rudnick edits discussion on *Rhythmic & Synthetic Processes in Growth* (Princeton Univ Press, 58, 224pp, $7.5).

February, 1959

A Quickie at Good New Books: J Adriani edits well-packed symposium on *The Recovery Room* (CC Thomas, Springfield, Ill, 1958, 132pp, $4.25). WB Bean well illustrates *Vascular Spiders & Related Lesions of the Skin* (CC Thomas, Springfield, Ill, 1958, 142pp, $8.5). M Bier edits important *Electrophoresis: Theory, Methods & Applications* (Academic Press, NY 3, 1959, 580pp, $15). H Brill introduces *Trifluoperazine: Clinical & Pharmacological Aspects* (Lea & Fibiger, Philadelphia 6, 1958, 219pp, $3.5). A Buhlmann discusses *Direkte Blutdruckmessung beim Menschen: Methoden & Ergebnisse im Korper- Lungenkreislauf* (Lange & Springer, West Berlin, 1958, 87pp, 20DM). EJM Campbell describes *The Respiratory Muscles & the Mechanics of Breathing* (Year Book Publ., Chicago 11, 1958, 131pp, $4.25). CF Diehl offers *Stuttering: A Compendium of Research & Theory* (CC Thomas, Springfield, Ill, 1958, 344pp, $10). A witty must is JK Galbraith's *The Affluent Society* (Houghton-Mifflin, Boston, 1958, 368pp, $5). Inspiring is WC Gibson's *Young Endeavor: Contributions to Science by Medical Students* (CC Thomas, Springfield, Ill, 1958, 312pp, $6.5). MH Harrower well correlates

clinical findings with projective tests in *Personality Change & Development as Measured by the Projective Tests* (Grune & Stratton, New York, 1958, 383pp, $10). HF Harlow & EN Woolsey edit *Biological & Biochemical Bases of Behavior* (Univ of Wis. Press, Madison, 1958, 476pp, $10). PH Hoch & J Zubin edit *Problems of Addiction & Habituation* (Grune & Stratton, New York, 1958, 250pp, $4.5). H Nowakowski edits *Hormone & Psyche: Die Endokrinologie des alternden Menschen* (Lange & Springer, W Berlin, 1958, 342pp, 70DM). M Rinkel edits symposium on *Chemical Concepts of Psychoses* (McDowell-Obolensky, NY, 1958, 485pp, $10). W Riese makes neat *History of Neurology* (MD Publ, New York, 1959, 223pp, $4). Excellent is JF Smithcors' *Evolution of the Veterinary Art* (Veterinary Medicine, Kansas City 2, Mo., 1959, 236pp, $6.5). AL Welsh reviews *Psychotherapeutic Drugs* (CC Thomas, Springfield, Ill, 1958, 144pp, $4.5). MG Wohl well edits *Long-Term Illness: Management of the Chronically Ill Patient* (WB Saunders, Philadelphia, 1959, 748pp, $15). GEW Wolstenholme & CM O'Connor edit *Amino Acids & Peptids with Antimetabolic Activity* (Little, Brown, Boston, 1958, 286pp, $8.75.

Last Year's Clear-up: A Arvay & L Balazsy find hypophysis responds to nervous stress by increased gonadotropin production (*Acta Physiol Acad Sci Hungar* 14/317/58). GK Benson shows reserpine inhibits mammary gland involution, decreases food intake & increases adrenal weight (*Proc Soc Exp Biol Med* 99/550/58). TC Butler edits talks on processes terminating drug action (*Fed Proc* 17/1158/58). LD Carlson opens talks on metabolic adaptations to cold (*ibid* 1044). H Davis introduces talks on neuropharmacology (*ibid* 1004). JM Dille edits *Proc West Pharmacol Sco* (1/1–55/58). E Endroczi & Co find new corticoids in human placenta (*Acta Physiol Acad Sci Hungar* 14/311/58). JC Kendrew is chief editor new *J Molecular Biol* issued by Academic Press, New York 3, $14 a volume. J Lear goes after "doctors & antibiotics" in re high pressure advertising (*Sat Rev* Jan 3/59). RB Pelton & RJ Williams find Capantothenate increases longevity on continued administration in rats (*Proc Soc Exp Biol Med* 99/632/58). A Phil & L Eldjarn discuss chemical protection to ionizing radiation in mammals (*Pharmacol Rev* 10/437/58). J Porszasz reports "spiractin" (1-piperidino-methyl-cyclohexane 2-on) is useful respiratory stimulant vs narcotic poisoning (*Acta Physiol Acad Hungar* 14/376/58). I Rosner indicates thyroxine may alter nervous receptor mechanisms (*Sechenov Physiol J USSR* 44/435/58). LH Snyder pays tribute to AE Garrod (*Lancet* 2/73, 214/1908) in reviewing rapid advance of medical genetics in re inborn errors of metabolism (*Science* 129/7/Jan 3/59). WG Spector discusses substances affecting capillary permeability (*Pharmacol Rev* 10/475/58). WM Stanley chairs symposium on cell culture (*Fed Proc* 17/967/58). OI Sukmanskii notes cutting vagosympathetic trunk reduces salivary excretion P^{32} on side of nerve cut (*Sechenov Physiol J USSR* 44/499/58). J Wessely notes that lymph flow decreases in shock but can be restored by dibenamine (*Acta Physiol Acad Sci Hungar* 14/327/58). EN Willmer discusses aspects of cellular differentiation (*Adv Sci* 15/95/58). AV Zhirmunskii finds muscle changes after denervation indicating development of parabiotic process (*Sechenov Physiol J USSR* 44/533/58).

Add to Last Year's Books: J Huxley's *Biological Aspects of Cancer* (Harcourt Brace, New York, 1958, 156pp, $3.75), & RI Lee's *A Doctor Speaks His Mind* (Little Brown, Boston, 1958, 120pp, $3).

C. D. LEAKE

MARCH, 1959

Biochemical: SW Fox gives overtones to thermal theory of biochemical origins (*AIBS Bull* 9/20/59). WD McElroy & B Glass edit *The Chemical Basis of Development* (Johns Hopkins Press, Baltimore, 59, 976pp, $15). H Netter surveys *Theoretische Biochemie* (Lange & Springer, Berlin-Wilmersdorf, 59, 800p, DM 86). R Steel edits *Biochemical Engineering* (Macmillan, NY 11, 59, 322pp, $7.5).

Social Pathology: H. Carter describes *The Angry Scar: The Story of Reconstruction* (Doubleday, NY, 59, 425pp, $6). P Drucker looks at *Landmarks of Tomorrow*, the consequences of the scientific explosion (Harper, NY, 59, 270pp, $4.5). J Lear pulls no punches in discussing certification of antibiotics (*Sat Rev* Feb 3/59, p43). TS Matthews says in *The Sugar Pill* that journalism's business is entertainment not news (Simon & Schuster, NY, 59, 227pp, $3.75; see also his remarks on who decides what's fit to print, *Sat Rev* Jan 24/59). MM Tumin considers *Desegregation: Resistance & Readiness*, Princeton Univ Press, 59, 270pp, $5).

Clinical: AB Dobkin finds no fixed acid accumulates in fluothane anesthesia (*Anesth* 20/10/59). EI Eger & HH Keasling find meprobamate as good as pentobarbital for anesthetic premedication (*ibid* p 1). TM Graham & Co show that prenatal irradiation impairs learning (*Proc Soc Exp Biol Med* 100/78/59). H Kalter & J Warkamy discuss metabolic production of congenital malformations by vitamin & hormone imbalances (*Physiol Rev* 39/69/59). RF Rushmer & OA Smith survey cardiac control (*ibid* p 41). JW St. Geme writes of melanin & melanomas, with 227 references (*J-Lancet* 79/82/59).

General: JB Conant reports wisely on *The American High School Today* (McGraw-Hill, NY, 59, 152pp, $3). A Garosi lauds *Siena della Storia della Medicina 1240–1555* (Olschki, Firenze, 59, 570pp, 15,000L). RH Major writes pleasantly on *Disease & Destiny & on Logan Clendening* (Univ Kansas Press, Lawrence, 59, 50pp, $2). W Pagel studies *Paracelsus* (S Karger, Basel, 59, 380pp, SwFr 70). JH Peck enjoys telling *All About Men* (Prentice-Hall, Englewood, NJ, 59, 275pp, $4). RO Steuer & JBdeCM Saunders review *Ancient Egyptian & Cnidian Medicine: The Relationship of Their Aetiological Concepts of Disease* (Univ of California Press, Berkeley, 59, 124pp, $3).

Preclinical: DM Aviado reviews cardiovascular effects of pressor amines (*Anesth* 20/71/59). ME Bierwagen & DL Smith note antithyroid action of reserpine (*Proc Soc Exp Biol Med* 100/108/59). MAB Brazier well edits 1st Macy Conference on *Central Nervous System & Behavior*, with pictorial survey of Russian CNS physiology, comments on Sechenov, Danilewsky, Wedensky, Ukhtomsky, Pavlov, Bechterev, Post-Pavlovian conditional reflexology, conditional reflexes & conditioned learning (Josiah Macy Foundation, NY, 59, 450pp, $5.25). HN Christensen reviews active transport of amino acids

(*Perspect Biol Med* 2/228/59). D Green & J Jarnefelt discuss enzymes & biological organization (*ibid* p 163). AS Gordon reviews hemopoietine (*Physiol Rev* 39/1/59). L Gross notes virus character of a radiation induced leukemia agent (*Proc Soc Exp Biol Med* 100/102/59). W Hild, KA Reiser & HJ Lehmann review *Das Neuron; Die Nervenzelle; Die Nervenfaser* (Lange & Springer, Berlin-Wilmersdorf, 59, *Ndb mikros Anat*, 4/4, 808pp, DM 318). FL Horsfall surveys new knowledge of viruses (*World-Wide Absts Gen Med*, Feb 59, p 11). JA Jacquez & Co find free ammonia in alveolar air (*Science* 129/269, 270, Jan 30/59). H Margenau discusses field theory in physics & biology (*Main Cur Med Thought* 15/58/59). A Nettleship considers protoplasm as a time-binding stream (*ibid* p 51). JM Renier notes *The Behavior of Enzyme Systems: An Analysis of Kinetics & Mechanisms* (Brugess, Minneapolis 15, 59, 210pp, $5). V Riley reports antitumor action of orthophenylenediamine (*Proc Soc Exp Biol Med* 100/155/59). JR Rubin & Co find increased beta-amino-isobutyric acid in urine of radiated humans (*ibid* p 130). D Rudnik edits *Developmental Cytology* (Ronald Press, NY 10, 59, 238pp, $7). PR Saunders finds toxic hypotensive peptide in stonefish venom (*Science* 129/272/Jan 30/59). RW Schayer analyses in vivo catabolism of histamine (*Physiol Rev* 39/116/59). LH Smith & Co find average life span of rat erythrocytes to be 65 days (*Proc Soc Exp Biol Med* 100/29/59). T Verhave notes effect of methamphetamine on operant level & avoidance behavior (*J Exp Anal Behav* 1/207/59). RM Warren & C Pfaffman note suppression of sweet taste by potassium gymnemate fr Indian *Gymnema sylvestre* leaves (*J Appl Physiol* 14/40/59). M Webb & FA Brown discuss timing of long cycle functional rhythms (*Physiol Rev* 39/127/59). W Wilbrandt reviews permeability & transport systems in living cells (*J Pharm Pharmacol* 11/65/59).

C. D. LEAKE